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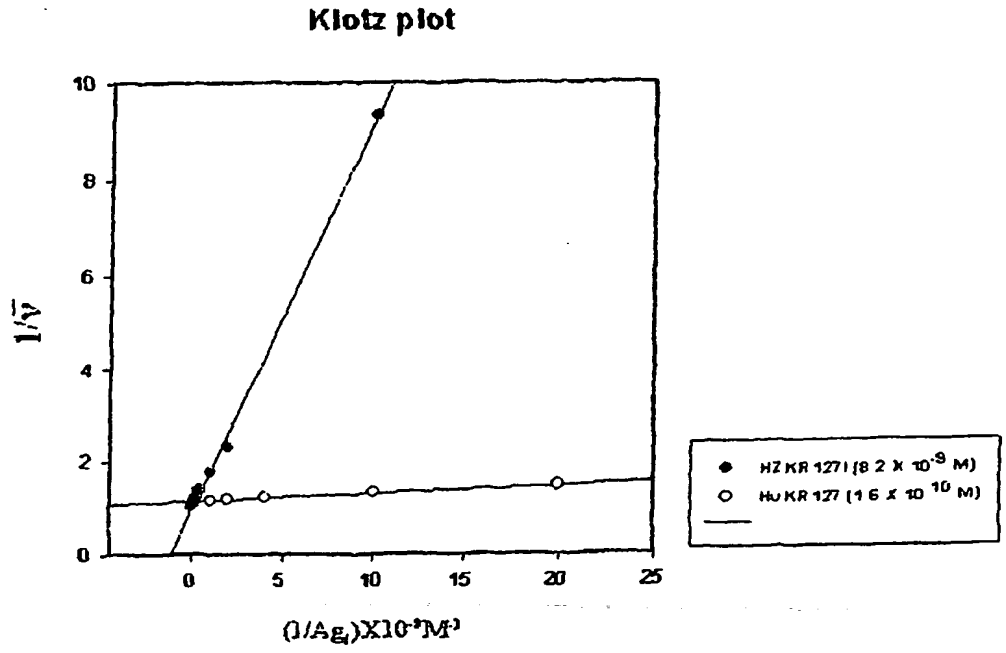
(54) Title: HUMANIZED ANTIBODY AND PROCESS FOR PREPARING SAME



(57) Abstract: A humanized antibody is produced by process comprising the steps of: (a) selecting a specificity determining residue (SDR) of the complementarity determining region (CDR) of murine monoclonal antibody heavy chain and light chain variable regions; and (b) grafting said SDR to at least one of the corresponding amino acid sequences in human antibody variable regions.

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Table 8b

antiboby	HzKR127I		HuKR127	
	peptide	MHC class II	peptide	MHC class II
	VGVYYCVQG	DRB1_0806	VGVYYCVQG	DRB1_0806
	IYLVSKLDS	DRB1_0301 DRB1_0305 DRB1_0306 DRB1_0307 DRB1_0308 DRB1_0309 DRB1_0311 DRB1_0405 DRB1_0410 DRB1_0801 DRB1_0802 DRB1_0804 DRB1_0806 DRB1_0813 DRB1_0817 DRB1_1101 DRB1_1102 DRB1_1104 DRB1_1106 DRB1_1107 DRB1_1114 DRB1_1120 DRB1_1121 DRB1_1128 DRB1_1301 DRB1_1302 DRB1_1304 DRB1_1305 DRB1_1307 DRB1_1311 DRB1_1321 DRB1_1322 DRB1_1323 DRB1_1327 DRB1_1328 DRB1_1501 DRB1_1506	IYLVSNRDS	DRB1_0402 DRB1_0404 DRB1_0405 DRB1_0408 DRB1_0410 DRB1_0423 DRB1_0804 DRB1_1102 DRB1_1104 DRB1_1106 DRB1_1114 DRB1_1121 DRB1_1301 DRB1_1307 DRB1_1311 DRB1_1322 DRB1_1323 DRB1_1327 DRB1_1328 DRB5_0101 DRB5_0105
	LIYLVSKLD	DRB1_0806 DRB1_1304 DRB1_1321	LIYLVSNRD	DRB1_0401 DRB1_0404 DRB1_0405 DRB1_0408 DRB1_0410 DRB1_0421 DRB1_0423 DRB1_0426 DRB1_1304
	YLVSKLDSG	0	YLVSNRDSG	DRB1_0309
total		106		40

As can be seen from Figs. 7 and 8, the number of the peptide sequence in the humanized antibody HuKR127 which binds to MHC class II was fewer than of that the HzKR127I. These results suggest that humanized antibody HuKR127 of the present invention is expected to reduce HAMA response to a greater extent than HzKR127I.

Table 8a

antiboby	HzkR127I		HuKR127	
	peptide	MHC class II	peptide	MHC class II
MHC class II -binding	ILMTQTPLS	DRB1_0301 DRB1_0305 DRB1_0306 DRB1_0307 DRB1_0308 DRB1_0309 DRB1_0311 DRB1_0401 DRB1_0402 DRB1_0404 DRB1_0405 DRB1_0408 DRB1_0410 DRB1_0421 DRB1_0423 DRB1_0426 DRB1_0804 DRB1_1101 DRB1_1102 DRB1_1104 DRB1_1106 DRB1_1107 DRB1_1114 DRB1_1121 DRB1_1128 DRB1_1301 DRB1_1304 DRB1_1305 DRB1_1307 DRB1_1311 DRB1_1321 DRB1_1322 DRB1_1323 DRB1_1327 DRB1_1328	IVMTQTPLS	0
	LMTQTPLSL	DRB1_0101 DRB1_0102 DRB1_1304	VMTQTPLSL	0
	WLLQKPGQS	DRB1_0101 DRB1_0305 DRB1_0309 DRB1_0401 DRB1_0408 DRB1_0421 DRB1_0426 DRB1_0802 DRB1_1101 DRB1_1107 DRB1_1114 DRB1_1120 DRB1_1128 DRB1_1302 DRB1_1305 DRB1_1307 DRB1_1321 DRB1_1323 DRB5_0101 DRB1_0105	WLLQKPGQP	0
	YYCVQGTHF	DRB1_0101 DRB1_0701 DRB1_0703 DRB5_0101 DRB5_0105	YYCVQGTHF	DRB1_0101 DRB1_0701 DRB1_0703 DRB5_0101 DRB5_0105
	YCVQGTHFP	DRB1_0401 DRB1_0421 DRB1_0426	YCVQGTHFP	DRB1_0401 DRB1_0421 DRB1_0426

Table 7

antiboby	HzKR127I		HuKR127	
	peptide	MHC class II	peptide	MHC class II
MHC class II -binding	LVQSGAEVV	DRB1_0306 DRB1_0307 DRB1_0308 DRB1_0311 DRB1_0421 DRB1_0701 DRB1_0703	LVQSGAEVK	0
	VKPGASVKV	DRB1_0102	KKPGASVKV	0
	FSSSWMNWV	DRB1_0703	FTSAWMNWV	0
	WIGRIYPGD	DRB1_0801 DRB1_0817	WMGRIYPSG	0
	FQGKATLTA	DRB1_0401 DRB1_0402 DRB1_0405 DRB1_0408 DRB1_0421 DRB1_0426 DRB1_0801 DRB1_0802 DRB1_0804 DRB1_0806 DRB1_0813 DRB1_0817 DRB1_1101 DRB1_1102 DRB1_1104 DRB1_1106 DRB1_1114 DRB1_1120 DRB1_1121 DRB1_1128 DRB1_1302 DRB1_1305 DRB1_1307 DRB1_1311 DRB1_1321 DRB1_1322 DRB1_1323	FQGRVTMTA	DRB1_0305 DRB1_0401 DRB1_0402 DRB1_0408 DRB1_0426 DRB1_0801 DRB1_0802 DRB1_0804 DRB1_0806 DRB1_0813 DRB1_0817 DRB1_1101 DRB1_1114 DRB1_1120 DRB1_1128 DRB1_1302 DRB1_1305 DRB1_1307 DRB1_1321 DRB1_1323 DRB1_1502
	YWGQGTLVT	DRB1_0401 DRB1_0405 DRB1_0421 DRB1_0426	RWGQGTLVT	0
	IGRIYPGDG	DRB5_0101 DRB5_0105	MGRIYPSGG	DRB1_0404 DRB1_0405 DRB1_0410 DRB1_0423
	YAQKFQGKA	DRB1_0802	YAQKFQGRV	0
	VYFCAREYD	DRB1_1304	VYYCAREYR	DRB1_0301
	YWGQGTLVT	DRB1_0401 DRB1_0405 DRB1_0421 DRB1_0426	RWGQGTLVT	0
total		50		26

561, 1999) to examine whether a peptide sequences which can bind to MHC (major histocompatibility complex) class II exists in the heavy and light chain variable regions of the humanized antibody.

Tables. 7 and 8 show the results of such analysis for MHC class II-binding peptide sequences in the heavy chain variable regions of HuKR127 and the light chain variable regions of HuKR127, respectively.

HUMANIZED ANTIBODY AND PROCESS FOR PREPARING SAME

Field of the Invention

The present invention relates to a process for preparing a humanized antibody by grafting SDRs (specificity determining residues) in CDRs (complementary determining residues) of murine monoclonal antibody to human antibody and the humanized antibody prepared according to said process.

Background of the Invention

For preventing infectious diseases such as hepatitis B, there has generally been used a method of administering immunoglobulins formed in blood plasma against a target antigen. However, the method has the problems that the immunoglobulins generally have low specificity and may contain contaminants.

Murine monoclonal antibody derived from mouse has been reported to have high affinity to antigen and is suitable for mass-production. However, repeated injection of murine monoclonal antibody induces an immune response because the murine antibody is regarded as a foreign antigen in humans (Shawler D.L. et al., *J. Immunol., 135*, 1530-1535(1985)).

Accordingly, numerous efforts have been made to generate "humanized antibody" by: grafting the CDR (complementarity determining region) of murine monoclonal antibody variable region which directly binds to antigens, to a human antibody framwork (CDR-grafting method); and replacing the amino acid residues of the human antibody framework region (FR) that influence the CDR conformation with the amino acid residues of murine monoclonal antibody. The humanized antibody thus obtained maintains the affinity and specificity of original murine monoclonal antibody, and minimizes HAMA(human anti-mouse antibody) response in humans (Riechmann et al., *Nature*, 332, 323-327(1988); Queen C. et al., *Proc. Natl. Acad. Sci. USA*, *86*, 10029-10033(1989); Nakatani et al., *Protein Engineering*, *7*, 435-443(1994)). However, this humanized antibody still causes problems when injected repeatedly into humans (Stephens et al., *Immunology*, *85*, 668-674(1995); Sharkey et al., *Cancer Research*, *55*, 5935s-5945s(1995)).

Approximately 300 millions of world population carry hepatitis B virus ("HBV") which may cause chronic infection, leading to cirrhosis and hepatocellular carcinoma (Tiollais P. and Buendia M.A., *Sci. Am., 264*, 48(1991)). The HBV envelope consists of three proteins, major protein containing S antigen, middle protein containing S and pre-S2 antigens, and large protein containing S, pre-S2 and pre-S1 antigens (Neurath A.R. and Kent S.B., *Adv. Vir. Res., 34*, 65-142(1988)). These surface antigens have been known to play important roles in the process of forming antibodies against HBV in hepatitis patient. The pre-S1 region, in particular, is found on infectious viral particles (Heermann et al., *J. Virol., 52*, 396-402(1984)) and plays a role in attachment to cell surface infection (Neurath et al., *Cell*, 46, 429(1986); Pontisso et al., *Virol.*, 173, 533, (1989); Neurath et al., *Vaccine*, *7*, 234(1989)). Thus a monoclonal antibody against the pre-S1 would be effective against viral infection.

The present inventors have previously reported a murine monoclonal antibody (KR127) against HBV pre-S1 (Korean Patent No. 246128), a murine monoclonal antibody KR127 gene encoding same (Korean Patent No. 250832) and a humanized antibody (HZKP127I) of KR127 prepared by CDR-grafting method (Korean Patent No. 246128).

The present inventors have further endeavored to develop a humanized antibody having minimized adverse immune response (HAMA response) as well as enhanced affinity to antigen, and found that HAMA response can be reduced when the amino acid residues of CDR of mouse antibody are replaced with those of human antibody.

Summary of the Invention

Accordingly, it is an object of the present invention to provide a process for preparing a humanized antibody which is expected to show lower HAMA response and has higher affinity than humanized antibody of the prior art.

It is another object of the present invention to provide a humanized antibody prepared according to said process.

It is a further another object of the present invention to provide a DNA encoding the heavy chain or light chain of said antibody and a vector comprising said DNA.

It is a still further object of the present invention to provide a

microorganism transformed with said vector.

In accordance with one aspect of the present invention, there is provided a process for preparing a humanized antibody comprising the steps of: (a) selecting a specificity determining residue (SDR) of the complementarity determining region (CDR) of murine monoclonal antibody heavy chain and light chain variable regions; and (b) grafting the amino acid residues of said SDR to at least one of the corresponding amino acid sequences in human antibody variable regions.

Brief Description of the Drawings

The above and other objects and features of the present invention will become apparent from the following description of the invention taken in conjunction with the following accompanying drawings; which respectively show:

Fig. 1 : the procedure for constructing an expression vector of a chimeric heavy chain;

Fig. 2 : the nucleotide and amino acid sequence of the humanized heavy chain variable region;

Fig. 3 : the procedure for constructing an expression vector of a chimeric light chain;

Fig. 4 : the nucleotide and amino acid sequence of the humanized light chain variable region;

Fig. 5 : the affinity to antigen of a humanized antibody having a heavy chain CDR mutant;

Fig. 6 : the procedure for constructing an expression vector of the humanized antibody; and

Figs. 7 and 8 : the results of analysis for MHC class II-binding peptide sequences in heavy chain variable regions of HuKR127 and light chain variable regions of HuKR127, respectively, which are compared with HzKR127I, respectively.

Detailed Description of the Invention

The humanized antibody of the present invention may be prepared by

grafting the amino acid residues of SDR of murine monoclonal antibody to the corresponding amino acid sequences in human antibody variable regions.

SDRs of the murine monoclonal antibody used in the present invention may be determined by independently replacing each amino acid residue of CDR of the murine monoclonal antibody with alanine, selecting transformants which have lower affinity (k_D) to antigen than the original murine antibody and determining the replaced CDR amino acid residues of said transformants as SDRs.

Further, in order to enhance the affinity to antigen, the CDR residues of a mouse antibody that increase the affinity and the framework residues that influence the conformation of CDR loops may also be grafted to the corresponding sites of human antibody.

For example, the present invention describes a process for preparing a humanized antibody for hepatitis B virus (HBV) pre-S1 by using murine monoclonal antibody KR127 (Korean Patent No. 250832) as follows:

After selecting SDR amino acid residues, which play important roles in binding with antigen, from CDR of the murine monoclonal antibody KR127 heavy and light chains, chimeric heavy chain and chimeric light chain genes may be prepared by combining either the variable region of KR127 antibody heavy chain with the constant region (C_γ 1) of human antibody or the variable region of KR127 antibody light chain with the constant region (C_κ) of human antibody.

SDRs of the murine monoclonal antibody for HBV pre-S1 are determined by replacing each amino acid residue of CDR HCDR1 (aa 31-35), HCDR2 (aa 50-65) and HCDR3 (aa 95-102) of the heavy chain (SEQ ID NO: 2) and CDR LCDR1 (aa 24-34), LCDR2(aa 50-56) and LCDR3(aa 89-97) of the light chain (SEQ ID NO: 4) of the murine monoclonal antibody KR127 with alanine according to the alanine scanning mutagenesis method and selecting the amino acid residues (SDRs) whose replacement with alanine bring about more than 3 times reduction in the affinity to antigen(K_D) as compared with the original murine antibody. Throughout this description, amino acid residue number is assigned according to Kabat numbering scheme (Kabat, E. A. et al, Sequences of Proteins of Immunological Interest. *National Institute of Health, Bethesda*, MD., 1991).

Examples of preferred SDR include tryptophan at position 33 (it is represented as "Trp33"), Met34, and Asn35 of HCDR1; Arg50, Tyr52, and

modified human heavy chain DP7-JH4 by Arg or Ala.

In addition, Ala71 and Lys73 in framework region 3 in the heavy chain variable region of KR127, which affects the conformation of the CDR loop, may further be grafted to human heavy chain DP7-JH4. Also, Leu36 and Arg46 in framework region 2 in the light chain variable region of KR127, which affects conformation of CDR loop, may be further grafted to human light chain DPH12-JK4.

The heavy chain variable region of humanized antibody of the present invention has the amino acid sequence of SEQ ID NO: 2, preferably encoded by the nucleotide sequence of SEQ ID NO: 1 and the inventive light chain variable region of humanized antibody has the amino acid sequence of SEQ ID NO: 4, preferably encoded by the nucleotide sequence of SEQ ID NO: 3.

The humanized antibody heavy chain and light chain of the present invention may be encoded by a gene comprising a nucleotide sequence deduced from the humanized antibody heavy chain and light chain according to the genetic code. It is known that several different codons encoding a specific amino acid may exist due to the codon degeneracy, and, therefore, the present invention includes in its scope all nucleotide sequences deduced from the humanized antibody heavy chain and light chain amino acid sequence. Preferably, the humanized antibody heavy chain and light chain gene sequences include one or more preferred codons of host cell.

The humanized antibody consisted of the humanized heavy chain HuKR127HC of the present invention and humanized light chain HZKR127I prepared by CDR-grafting has an affinity to antigen of about over 50 times higher than that of the humanized antibody HZKR127I.

The humanized antibody consisting of the humanized heavy chain HuKR127KC of the present invention and humanized light chain HZKR127I prepared by CDR-grafting has an affinity to antigen equal to that of the humanized antibody HZKR127I.

The genes of humanized antibody heavy chain and light chain thus prepared may be inserted to pdCMV-dhfrC-HAV6 vector (KCTC 10028BP) to obtain an expression vector pdCMV-dhfrC-HuKR127 which can express both humanized antibody heavy chain HuKR127HC and light chain HZKR127I. The expression vector of the present invention may be introduced into microorganism, e.g., *E. coli* DH5α according to a

Pro52a of HCDR2; Glu95, Tyr96, and Glu98 of HCDR3 of the murine monoclonal antibody KR127 heavy chain; Leu27b, Tyr27d, Ser27e; Asn28, Lys30, Tyr32, and Asn34 of LCDR1; Leu50 and Asp55 of LCDR2; and Val89, Gln90, Gly91, Thr92, His93, Phe94, Pro95, and Gln96 of LCDR3 of the murine monoclonal antibody KR127 light chain.

The humanized antibody of the present invention can be prepared by grafting one or more SDRs determined as above onto the human antibody heavy chain and/or light chain. The human antibody heavy chain which may be used in the present invention is human heavy chain DP7-JH4 consisting of human immunoglobulin germline VH gene segment DP7 (Tomlinson et al., *J. Mol. Biol.*, *227*, 776-798, 1992) and JH4 segment (Ravetch et al., *Cell*, *27*, 583-591, 1981). The human antibody light chain which may be used in the present invention is human light chain DPK12-JH4 consisting of human immunoglobulin germline VK gene segment DPK12 (Cox et al., *Eur. J. Immunol.*, *24*, 827-836 (1994)) and JH4 segment (Hieter et al., *J. Biol. Chem.*, *257*, 1516-1522 (1982)).

The humanized heavy chain of the present invention may be prepared by grafting at least one of Trp33, Met34, and Asn35 of HCDR1; Arg50, Tyr52, and Pro52a of HCDR2; Glu95, Tyr96, and Glu98 of HCDR3 of the murine monoclonal antibody KR127 heavy chain to the corresponding amino acid sequences in human antibody heavy chain. The inventive humanized light chain may be prepared by grafting at least one of Leu27b, Tyr27d, Ser27e; Asn28, Lys30, Tyr32, and Asn34 of LCDR1; Leu50 and Asp55 of LCDR2; and Val89, Gln90, Gly91, Thr92, His93, Phe94, Pro95, and Gln96 of LCDR3 of the murine monoclonal antibody KR127 light chain to the corresponding amino acid sequences in human antibody light chain DPH12-JK4.

Moreover, the affinity to antigen of the humanized antibody can be enhanced by the follow substitutions:

(a) the amino acid residue at position 32 in HCDR1 of the modified human heavy chain DP7-JH4 by Ala;

(b) the amino acid residue at position 97 in HCDR3 of the modified human heavy chain DP7-JH4 by Arg or Ala;

(c) the amino acid residue at position98 in HCDR3 of the modified human heavy chain DP7-JH4 by Val; and

(d) the amino acid residue at position 102 in HCDR3 of the

conventional transformation method to obtain transformants E. coli DH5α / pdCMV-dhfrC-HuKR127. The transformants *E. coli* DH5α / pdCMV-dhfrC-HuKR127 was deposited on March 13, 2002 with the Korean Collection for Type Cultures(KCTC)(Address: Korea Research Institute of Bioscience and Biotechnology(KRIBB), #52, Oun-dong, Yusong-ku, Taejon, 305-333, Republic of Korea) under the accession number, KCTC 10198BP, in accordance with the terms of Budapest Treaty on the International Recognition of the Deposit of Microorganism for the Purpose of Patent Procedure.

Meanwhile, CHO/HuKR127, CHO (Chinese hamster ovary) cell line transfected with vector pdCMV-dhfrC-HuKR127, was deposited on March 13, 2002 with the Korean Collection for Type Cultures(KCTC) under the accession number, KCTC 10199BP, in accordance with the terms of Budapest Treaty on the International Recognition of the Deposit of Microorganism for the Purpose of Patent Procedure.

The humanized antibody HuKR127 of the present invention produced by culturing the CHO/HuKR127 cell line has a higher affinity to antigen and is expected to reduce HAMA (human anti-mouse antibody) response to a greater extent than the conventional antibody prepared according to the CDR-grafting method.

Accordingly, the humanized antibody of the present invention can be used in preventing hepatitis B virus infection and treating chronic Hepatitis B.

Thus, for preventing hepatitis B virus infection and treating chronic Hepatitis B, a pharmaceutical formulation of the inventive humanized antibody may be prepared in accordance with any of the conventional procedures.

The pharmaceutical composition of the present invention can be administered via various routes including intravenous and intramuscular introduction. It should be understood that the amount of the active ingredient actually administered ought to be determined in light of various relevant factors including the condition to be treated, the chosen route of administration, the age, sex and body weight of the individual patient, and the severity of the patient's symptom; and, therefore, the above dose should not be intended to limit the scope of the invention in any way.

The following Examples are intended to further illustrate the present invention without limiting its scope.

Example 1: Preparation of mouse/human chimeric heavy chain gene

The gene encoding leader sequence and the γ1 constant region of the human antibody heavy chain were separately prepared by carrying out PCR using pCMV-HKR127HC (Korean Patent No. 246128, KCTC 0531BP) as a template and a primer set of Ryu94 (SEQ ID NO: 5) and HUR43-1 (SEQ ID NO: 6) or HUR46-1 (SEQ ID NO: 9) and HUR31(SEQ ID NO: 10).

The gene encoding heavy chain variable region of the murine monoclonal antibody KR127 was prepared by carrying out PCR using pKR127H(Korean Patent No. 250832, KCTC 0333BP) as a template and primers HUR44-1(SEQ ID NO: 7) and HUR45-1(SEQ ID NO: 8).

Ryu94: 5'-GAG AAT TCA CAT TCA CGA TGT ACT TG-3'
HUR43-1: 5'-CTG CTG CAG CTG GAC CTG ACT CTG GAC ACC ATT-3'
HUR44-1: 5'-CAG GTC CAG CTG CAG CAG TCT GGA CCT GAA CTG-3'
HUR45-1: 5'-TGG GCC CTT GGT GGA GGC TGC AGA GAC AGTGAC-3'
HUR46-1: 5'-GCC TCC ACC AAG GGC CCA TCG GTC TTC CCC CTG-3'
HUR31: 5'-CAG CGG CCG CTC ATT TAC CCG GGG ACA G-3'

Each PCR reaction was carried out using 10 ng of template, 1 μl of each primer (50 ppm), 0.5 μl of *Pfu* DNA polymerase (Promega), 4 μl of 2.5 mM dNTPmix and 5 μl of 10 x Pfu reaction buffer solution. After pre-denaturation at 95℃ for 5 minutes, a PCR cycle was repeated 25 times, the cycle being composed of: 95℃ for 30 sec., 50℃ for 30 sec. and 72℃ for 45 sec. After annealing the DNA fragment obtained by using primers Ryu94 and HUR43-1, the DNA fragment obtained by using primers HUR44-1 and HUR45-1, and the DNA fragment obtained by using primers HUR46-1 and HUR31 were recombined by conducting recombinant PCR using primers Ryu94 and HUR31. The recombinant PCR reaction was carried out using the same reaction buffer solution as used above. After pre-denaturation

at 95℃ for 5 minutes, a PCR cycle was repeated 30 times, the cycle being composed of: 95℃ for 30 sec., 50℃ for 30 sec. and 72℃ for 60 sec., and finally, the extension reaction was carried out at 72℃ for 5 min.

The chimeric heavy chain gene thus prepared was cleaved with *Eco*RI(GAATTC) and *Nde*I (GCGGCCGC) and inserted at the *Eco*RI/*Nde*I section of vector pcDdA (plasmid which is removed ApaI site in the multiple cloning site of pcDNA received from Invitrogen), to obtain vector pcDdAchKR127HC (Fig. 1). The base sequence of the chimeric heavy chain variable region gene (KR127VH) was confirmed by DNA sequence analysis (Fig. 2).

Example 2: Preparation of mouse/human chimeric light chain gene

The gene encoding reader sequence and the constant region of the human antibody light chain were each prepared by carrying out PCR using pKC-dhfr-HKR127 (Korean Patent No. 2000-33008, KCTC 0529BP) as a template and a primer set of Ryu86 (SEQ ID NO: 11) and HUR48 (SEQ ID NO: 12) or HUR51 (SEQ ID NO: 15) and CK1D (SEQ ID NO: 16).

The gene encoding light chain variable region of the murine monoclonal antibody KR127 was prepared by carrying out PCR using pKR127K (Korean Patent No. 250832, KCTC 0334BP) as a template and primers HUR49 (SEQ ID NO: 13) and HUR50 (SEQ ID NO: 14).

Ryu86: 5'-CAA AGC TTG GAA GCA AGA TGG ATT CA-3'
HUR48: 5'-CAA GAT ATC CCC ACA GGT ACC AGA TAC-3'
HUR49: 5'-TGT GGG GAT ATC TTG ATG ACC CAA ACT-3'
HUR50: 5'-CAC AGA TCT TTT GAT TTC CAG CTT GGT-3'
HUR51: 5'-ATC AAA AGA TCT GTG GCT GCA CCA TCT-3'
CK1D: 5'-GCG CCG TCT AGA ATT AAC ACT CTC CCC TGT TGA AGC TCT TTG TGA CGG GCG AACTCAG-3'

Each PCR reaction was carried out according to the method described in Example 1 except that primers Ryu86 and CK1D were used to ligate the annealed DNA fragments obtained by PCR reactions.

The chimeric light chain gene thus prepared was cleaved with *Hin*dIII (AAGCTT) and *Xba*I (TCTAGA) and inserted at the *Hin*dIII/*Xba*I

section of vector pBluescript KS, to obtain a recombinant plasmid. Subsequently, the recombinant plasmind was cleaved with *Hin*dIII and *Apa*I and inserted at the *Hin*dIII/*Apa*I section of vector pCMV-dhfr (KCTC 8671P), to obtain plasmid pKC-dhfr-chKR127(Fig. 3). The base sequence of the chimeric light chain varible region gene (KR127VK) was confirmed by DNA sequence analysis (Fig, 4).

Example 3: Mutation of CDR of chimeric KR127 antibody heavy chain by alanine injection

To examine whether each amino acid residue of KR127 heavy chain HCDR1 (aa 31-35), HCDR2(aa 50-65) and HCDR3 (aa 95-102) binds to antigen, PCR reaction was carried out using vector pcDdA-chKR127HC as a template to prepare a modified gene, wherein an amino acid residue of CDR was replaced with alanine (the replaced amino acid residue No. was indicated as Kabat number) (see Fig. 2).

A forward primer YM001N of SEQ ID NO: 17 was designed to provide the sequence corresponding to the reader sequence at the 5'-end of the chimeric heavy chain gene and *Eco*RI restrition site, and a reverse primer YM003 of SEQ ID NO: 18 was designed to have the sequence corresponding to the N-terminal downstream of CH1 domain of human heavy chain gene and ApaI restriction site.

YM001N: 5'-CCG GAA TTC ACA TTC ACG ATG TAC TTG-3'
YM003: 5'-TGC CCC CAG AGG TGC T-3'

The 5'-end primer ym257 of SEQ ID NO: 19 (corresponding to nucleotide Nos. 80 to 112 of SEQ ID NO: 1) was designed to replace Ser31 of HCDR1 with alanine (S31A) and the 3'-end primer YM258 of SEQ ID NO: 20 (corresponding to nucleotide Nos. 101 to 71 of SEQ ID NO: 1), to replace AGT (coding for Ser) of nucleotide Nos. 91 to 93 of HCDRI gene with GCT (coding for alanine).

Each PCR reaction was carried out according to the method described in Example 1 except that primer sets, YM001N and YM258; and ym258 and YM003, were used and also that primers YM001N and YM003 were used to recombine the annealed DNA fragments obtained by PCR.

The chimeric light chain gene thus prepared was cleaved with *EcoR*I and *Apa*I and inserted at the *EcoRI/Apa*I section of vector pcDdA-chKR127HC prepared in Example 1, to obtain pcDdA-chKR127HC-S31A. The base sequence of the humanized antibody heavy chain variable region gene was confirmed by DNA sequence analysis. Vectors containing mutants thus prepared are shown in Table 1.

In Table 1, primer and mutation positions are numbered based on the base sequence of SEQ ID NO: 1.

Table 1

CDR	primer		primer position	mutation position	mutant	vector
HCDR1	F	ym257	80-112	91-93	Ser(AGT)→ Ala(GCT)	pcDdA-chKR127HC-S31A
	R	YM258	101-71			
	F	ym259	83-112	94-96	Ser(TCT)→ Ala(GCT)	pcDdA-chKR127HC-S32A
	R	YM260	106-73			
	F	ym261	86-117	97-99	Trp(TGG)→ Ala(GCG)	pcDdA-chKR127HC-W33A
	R	YM262	108-76			
	F	ym263	90-118	100-102	Met(ATG)→ Ala(GCG)	pcDdA-chKR127HC-M33A
	R	YM264	111-79			
	F	ym265	94-120	103-105	Asn(AAC)→ Ala(GCC)	pcDdA-chKR127HC-N35A
	R	ym266	112-81			
HCDR2	F	YM221	139-174	148-150	Arg(CGG)→ Ala(GCC)	pcDdA-chKR127HC-R50A
	R	YM222	158-128			
	F	YM225	143-178	151-153	Ile(ATT)→ Ala(GCT)	pcDdA-chKR127HC-I51A
	R	YM226	162-131			
	F	YM227	145-180	154-156	Tyr(TAT)→ Ala(GCT)	pcDdA-chKR127HC-Y52A
	R	YM228	165-135			
	F	ym229	148-181	157-159	Pro(CCT)→ Ala(GCT)	pcDdA-chKR127HC-P52aA
	R	YM230	167-136			
	F	ym231	150-186	160-162	Gly(GGA)→ Ala(GCA)	pcDdA-chKR127HC-G53A
	R	YM232	173-145			
	F	ym233	152-188	163-165	Asp(GAT)→ Ala(GCT)	pcDdA-chKR127HC-D54A
	R	YM234	176-144			
	F	ym235	155-193	166-168	Gly(GGA)→ Ala(GCA)	pcDdA-chKR127HC-G55A
	R	YM236	178-146			
	F	ym237	158-194	169-171	Asp(GAT)→ Ala(GCT)	pcDdA-chKR127HC-D56A
	R	ym238	184-149			
	F	ym239	160-195	172-174	Thr(ACT)→ Ala(GCT)	pcDdA-chKR127HC-T57A
	R	ym240	185-150			
	F	ym241	164-196	175-177	Asn(AAC)→ Ala(GCC)	pcDdA-chKR127HC-N58A
	R	ym242	187-150			
HCDR3	F	YM207	286-317	295-297	Glu(GAG)→ Ala(GCG)	pcDdA-chKR127HC-E95A
	R	YM208	305-274			
	F	YM209	289-316	298-300	Tyr(TAC)→ Ala(GCC)	pcDdA-chKR127HC-Y96A
	R	YM210	307-276			
	F	YM211	292-318	301-303	Asp(GAC)→ Ala(GCC)	pcDdA-chKR127HC-D97A
	R	YM212	313-279			
	F	YM213	296-321	304-306	Glu(GAG)→ Ala(GCG)	pcDdA-chKR127HC-E98A
	R	YM214	315-285			
	F	YM255	303-327	310-312	Tyr(TAC)→ Ala(GGC)	pcDdA-chKR127HC-Y102A
	R	YM256	319-289			

Test Example 1: Expression of chimeric antibody having a modified heavy chain and its affinity to antigen

(step 1) Expression of chimeric antibody

COS7 cells (ATCC CRL-1651) were seeded to DMEM media (GIBCO) containing 10% bovine serum and subcultured in an incubator at 37°C under an atmosphere of 5% CO_2. 1 x 10^6 cells thus obtained were seeded to the same media and incubated at 37°C overnight. Thus, 5 μg of plasmid pKC-dhfr-chKR127 (expressing chimeric light chain) obtained in Example 2, 5 μg of plasmid obtained in Example 3 were diluted with OPTI-MEMI(GIBCO) to 800 $\mu\ell$. 50 $\mu\ell$ of Lipofectamine (GIBCO) were diluted with the same solution to 800 $\mu\ell$. The resulting solutions were added to a 15 $m\ell$ tube, mixed and then, kept at room temperature for more than 15 minutes. Meanwhile, COS7 cells incubated as above were washed three times with OPTI-MEM I. Then, 6.4 $m\ell$ of OPTI-MEM I was added to the DNA-Lipofectamine mixture and the resulting solution was evenly distributed on the COS7 cells, which were cultured for 48 hours in a 5% CO_2 incubator to obtain a supernatant. The resulting solution was subjected to sandwich ELISA analysis using anti-human IgG (Sigma) as a capture antibody and anti-human antigen (Fc-specific)-horseradish peroxidase (PIERCE) as a secondary antibody to confirm the expression of the chimeric antibody.

(step 2) Affinity to antigen

150 ng of HBV recombinant antigen GST-pre-S1(1-56) (H. S. Kim and H. J. Hong, *Biotechnology Letters, 17,* 871-876(1995)) was coated to each well of a microplate and 5 ng of the supernatant obtained in Step 1 was added to each well. The resulting solution was subjected to indirect ELISA using the same secondary antibody as used in step 1, followed by measuring the absorbance at 450 nm. Further, the affinity to antigen (K_D) of each modified heavy chain was determined by competitive ELISA method (Ryu et al., *J. Med. Virol., 52,* 226(1997)) and compared with that of pCK-dhfr-chKR127 containing wildtype chimeric heavy chain. The result is shown in Table 2.

Table 2

CDR	Mutant	K_D (nM)
	WT	11.0 ± 1.664
H1	S31A	14.67 ± 2.386
	S32A	8.455 ± 0.840
	W33A	>10000
	M34A	>10000
	N35A	>10000
H2	R50A	>10000
	I51A	12.8 ± 1.05
	Y52A	276.8 ± 23.60
	P52aA	170.3 ± 5.318
	G53A	7.697 ± 0.980
	D54A	1.663 ± 0.477
	G55A	5.766 ± 0.211
	D56A	6.59 ± 1.09
	T57A	13.68 ± 4.016
	N58A	1.568 ± 0.085
H3	E95A	>10000
	Y96A	>10000
	D97A	0.57 ± 0.03
	E98A	64.2 ± 7.78
	Y102A	3.581 ± 0.457

As shown in Table 2, the affinities to antigen of the mutants obtained by replacing Trp33, Met34, or Asn35 of HCDR1; Arg50, Tyr52, or Pro52a of HCDR2; Glu95, Tyr96, or Glu98 of HCDR3 with alanine were more than 3 times lower than that of wild type. However, a mutant having alanine substituting for Asp97 or Tyr102 residue of HCDR3 exhibited an enhanced affinity to antigen.

Example 4: Preparation of HCDR3 mutants and their affinities to antigen

(step 1) D97R and E98V mutants

Each mutant was prepared by replacing Asp97 or Glu98 of HCDR3 with arginine as a positively charged amino acid (it is represented as "D97R") or valine as a neutral amino acid (it is represented as "E98V") according to the site-directed mutagenesis as used in Example 3. Vectors containing mutants prepared as above are shown in Table 3.

Table 3

CDR	primer		primer position	mutation position	mutant	vector
HCDR3	R	P1	312-279	301-303	Asp(GAC)→ Arg(CGG)	pcDdA-chKR127HC-D97R
	F	P2	295-326			
	R	P3	312-279	301-303	Asp(GAC)→ Val(GTT)	pcDdA-chKR127HC-D97V
	F	P4	295-326			
	R	P5	312-279	304-306	Glu(GAG)→ Arg(CGG	pcDdA-chKR127HC-E98R
	F	P6	295-326			
	R	P7	312-279	304-306	Glu(GAG)→ Val(GTT)	pcDdA-chKR127HC-E98V
	F	P8	295-326			

Then, each mutant thus obtained was measured for its affinity to antigen in according to the method described in Test Example 1 and compared with that of the wild type.

As shown in Fig 5, the affinity to antigen of D97R was more than 3 times higher than that of the wild type, which the affinity to antigen of E98V, more than 4 times higher than that of the wild type. However, mutant E98R showed a low affinity to antigen.

(Step 2) D97R/E98V mutant

To prepare D97R/E98V mutant containing both D97R and E98V, which were found to be mutants having high affinity to antigen, PCR reaction was carried out using pcDdA-chKR127HC-D97R which contains D97R gene as a template and primers P7 and P8.

Then, the D97R/E98V mutant thus obtained was measured for its affinity to antigen in according to the method described in Test Example 1.

As shown in Fig 5, the affinity to antigen of D97R/E98V was more than 15 times higher than that of the wild type.

(Step 3) D97R/E98V/Y102A mutant

To prepare D97R/E98V/Y102A mutant containing D97R, E98V and Y102A, PCR reaction was carried out using pcDdA-chKR127HC-RV containing D97R/E98V as a template and primers YM255 and YM256.

Then, the D97R/E98V/Y102A mutant (hereinafter "RVAA") thus

obtained was measured for its affinity to antigen in according to the method described in Test Example 1.

As shown in Fig 5, the affinity to antigen of D97R/E98V/Y102A was similar to that of D97R/E98V.

(Step 4) D97R/E98V/Y102E and D97R/E98V/Y102R mutants

To prepare D97R/E98V/Y102E mutant and D97R/E98V/Y102R mutant, PCR reaction was carried out using pcDdA-chKR127HC-RV containing D97R/E98V as a template, and primer sets P17/P18 and P19/P20, respectively.

Vctor containing mutants prepared above are shown in Table 4.

Table 4

	primer		primer position	mutation position	mutant	vector
HCDR3	R	P17	312-279	307-309	Tyr(TAC)→ Glu(GAG)	pcDdA-chKR127HC-RVAE
	F	P18	295-326			
	R	P19	312-279	307-309	Tyr(TAC)→ Arg(CGT)	pcDdA-chKR127HC-RVAR
	F	P20	295-326			

Then, D97R/E98V/Y102E mutant (hereinafter "RVAE") and D97R/E98V/Y102R mutant (hereinafter "RVAR") thus obtained were measured for respective affinities to antigen in according to the method described in Test Example 1.

As shown in Fig 5, the affinity to antigen of RVAE was similar to that of RVAA, while the affinity to antigen of RVAR was higher than that of RVAA.

Test Example 2: Measurement of affinity to antigen of RVAR

The RVAR mutant prepared in step 4 of Example 4 was subjected to competitive ELISA to measure its affinity to antigen as follows:

COS7 cells were transfected with the plasmid prepared in step 4 of Example 4 and the plasmid expressing chimeric light chain(pKC-dhfr-chKR127) prepared in Example 2 to produce an antibody. 5 ng of the antibody thus obtained was reacted with pre-S1 antigen (1×10^{-7} to 1×10^{-12}

M) at 37 ℃ for 2 hours. The resulting solution was added to each well of a 96-well microplate coated with pre-S1 antigen and reacted at 37℃ for 30 minutes, and then the resulting solution was subjected to ELISA analysis according to the method described in Example 4. Used as a control is chimeric antibody (chKR127) obtained from COS7 cells transfected with pcDdA-chKR127HC and pKC-dhfr-chKR127.

The affinity to antigen of RVAR was about 1.8×10^{-10} M, which is 45 times higher than that of chKR127, about 8.2×10^{-9} M

Example 5: Mutation of CDR of chimeric KR127 antibody light chain by alanine injection

To examine the affinity of each amino acid residue of KR127 light chain LCDR1 (aa 24-34), LCDR2(aa 50-60) and LCDR3 (aa 89-97) to antigen, PCR reaction was carried out using vector pKC-dhfr-chKR127 as a template to prepare a modified gene having each amino acid residue of CDR replaced with alanine (the replaced amino acid residue Number was indicated as Kabat number)(see Fig. 2).

Forward primer YM004 of SEQ ID NO: 21 was designed to provide the sequence corresponding to the reader sequence at the 5'-end of the chimeric light chain gene and the *Hin*dIII restrition site, and a reverse primer YM009 of SEQ ID NO: 22 was designed to have the sequence corresponding to the N-terminal region of human light chain gene and the *Bsi*WI(CGTACG) restriction site. These primers were used in preparation of mutants of light chain CDR residue.

YM004: 5'-CCA AAG CTT GGA AAG ATG GAT TCA CAG-3'
YM009: 5'-GCA GCC ACC GTA CGT TTG ATT TCC ACC TTG GT-3'

Forward primer YM135 was designed to replace Ser26 of LCDR1 with alanine (S26A) and a reverse primer YM136, to replace AGT coding for Ser at the nucleotide Nos. 76 to 78 of LCDRI gene with GCT coding for alanine.

PCR reactions were carried out according to the method described in Example 1 except that primer sets, YM004/YM135, and YM136/YM009, were used and that primers YM004 and YM009 were used to recombine the annealed DNA fragments obtained by PCR.

The variable region gene of the mutant thus prepared was cleaved with *Hind*III and *Bsi*WI and inserted at the *Hind*III/*Bsi*WI section of vector pKC-dhfr-chKR127, to obtain pKC-dhfr-chKR127BS-S26A. The base sequence of the modified chimeric light chain variable region gene was confirmed by DNA sequence analysis. The vectors containing mutants prepared above are shown in Table 5.

In Table 5, the primer and mutation positions are numbered based on the base sequence of SEQ ID NO: 3.

Table 5

	primer		primer position	mutation position	mutant	vector
LCDR1	F	YM135	67-102	76-78	Ser(AGT)-Ala(GCT)	pKC-dhfr-chKR127BS-S26A
	R	YM136	86-54			
	F	YM137	69-107	79-81	Gln(CAG)-Ala(GCG)	pKC-dhfr-chKR127BS-Q27A
	R	YM138	91-56			
	F	YM139	70-111	82-84	Ser(AGC)-Ala(GCC)	pKC-dhfr-chKR127BS-S27aA
	R	YM140	94-58			
	F	YM141	73-114	85-87	Leu(CTC)-Ala(GCC)	pKC-dhfr-chKR127BS-L27bA
	R	YM142	98-64			
	F	YM143	73-116	88-91	Leu(TTA)-Ala(GCA)	pKC-dhfr-chKR127BS-L27cA
	R	YM144	102-68			
	F	YM145	79-118	91-93	Tyr(TAT)-Ala(GCT)	pKC-dhfr-chKR127BS-Y27dA
	R	YM146	103-69			
	F	YM147	83-119	94-96	Ser(AGT)-Ala(GCT)	pKC-dhfr-chKR127BS-S27eA
	R	YM148	107-69			
	F	YM149	84-120	97-99	Asn(AAT)-Ala(GCT)	pKC-dhfr-chKR127BS-N28A
	R	YM150	110-70			
	F	YM151	88-127	100-102	Gly(GGA)-Ala(GCA)	pKC-dhfr-chKR127BS-G29A
	R	YM152	114-74			
	F	YM153	91-130	103-105	Lys(AAA)-Ala(GCA)	pKC-dhfr-chKR127BS-K30A
	R	YM154	116-77			
	F	YM155	93-132	106-108	Thr(ACC)-Ala(GCC)	pKC-dhfr-chKR127BS-T31A
	R	YM156	118-80			
	F	YM103	99-133	109-111	Tyr(TAT)-Ala(GCT)	pKC-dhfr-chKR127BS-Y32A
	R	YM104	120-83			
	F	N34A-F	106-132	115-118	Asn (AAT)-Ala(GCT)	pKC-dhfr-chKR127BS-Y34A
	R	N34A-R	126-100			

	primer		primer position	mutation position	mutant	vector
LCDR2	F	YM129	151-188	163-165	Leu(CTG)-Ala(GCG)	pKC-dhfr-chKR127BS-L50A
	R	YM130	175-140			
	F	YM131	153-191	166-168	Val(GTG)-Ala(GCG)	pKC-dhfr-chKR127BS-V51A
	R	YM132	179-145			
	F	YM133	157-192	169-171	Ser(TCT)-Ala(GCT)	pKC-dhfr-chKR127BS-S52A
	R	YM134	181-147			
	F	K53A-F	163-187	172-174	Lys(AAA)-Ala(GCA)	pKC-dhfr-chKR127BS-K53A
	R	K53A-R	178-154			
	F	L54A-F	163-189	175-177	Leu(CTG)-Ala(GCG)	pKC-dhfr-chKR127BS-L54A
	R	L54A-R	180-159			
	F	D55A-F	170-195	178-180	Asp(GAC)-Ala(GCC)	pKC-dhfr-chKR127BS-D55A
	R	D55A-R	184-163			
	F	K56A-F	175-198	181-183	Ser(TCT)-Ala(GCT)	pKC-dhfr-chKR127BS-S56A
	R	K56A-R	190-168			
LCDR3	F	YM113	270-304	280-282	Val(GTG)-Ala(GCG)	pKC-dhfr-chKR127BS-V89A
	R	YM114	292-258			
	F	YM115	274-307	283-285	Gln(CAA)-Ala(GCA)	pKC-dhfr-chKR127BS-Q90A
	R	YM116	294-259			
	F	YM117	277-310	286-288	Gly(GGT)-Ala(GCT)	pKC-dhfr-chKR127BS-G91A
	R	YM118	296-265			
	F	YM119	281-310	289-291	Thr(ACA)-Ala(GCA)	pKC-dhfr-chKR127BS-T92A
	R	YM120	302-266			
	F	YM121	282-313	292-294	His(CAT)-Ala(GCT)	pKC-dhfr-chKR127BS-H93A
	R	YM122	304-271			
	F	YM111	286-314	295-297	Phe(TTT)-Ala(GCT)	pKC-dhfr-chKR127BS-F94A
	R	YM112	307-274			
	F	YM123	286-317	298-300	Pro(CCT)-Ala(GCT)	pKC-dhfr-chKR127BS-P95A
	R	YM124	308-278			
	F	YM125	292-319	301-303	Gln(CAG)-Ala(GCG)	pKC-dhfr-chKR127BS-Q96A
	R	YM126	311-279			
	F	YM127	294-320	304-306	Thr(ACG)-Ala(GCG)	pKC-dhfr-chKR127BS-T97A
	R	YM128	313-282			

Test Example 3: Measurement of affinity to antigen of light chain mutant

COS7 cell was transfected with each of the light chain mutants prepared in Example 5 and the plasmid expressing chimeric heavy chain(pcDdA-chKR127HC) to produce an antibody. The antibody obtained

was measured for its affinity to antigen in accordance with the method described in Test Example 1.

Table 6 shows the results obtained for the mutants and pdDA-chKR127HC containing wildtype chimeric KR127 heavy chain.

Table 6

CDR	mutant	K_D (nM)
L1	S26A	6.49 ± 0.244
	Q27A	14.2 ± 2.29
	S27aA	37.9 ± 6.66
	L27bA	>10000
	L27cA	36.8 ± 11.01
	Y27dA	1032.7 ± 56.1
	S27eA	>10000
	N28A	>10000
	G29A	23.94 ± 2.62
	K30A	>10000
	T31A	13.19 ± 1.98
	Y32A	>10000
	N34A	>10000
L2	L50A	159.4 ± 21.37
	V51A	37.00 ± 10.33
	S52A	14.08 ± 0.509
	K53A	7.928 ± 0.976
	L54A	12.57 ± 2.453
	D55A	225.2 ± 2.970
	S56A	12.95 ± 0.367
L3	V89A	121.2 ± 4.62
	Q90A	>10000
	G91A	>10000
	T92A	74.2 ± 2.90
	H93A	54.5 ± 4.48
	F94A	>10000
	P95A	>10000
	Q96A	293.6 ± 7.13
	T97A	17.3 ± 2.56

As shown in Table 6, the affinities to antigen of the mutants obtained by replacing the Leu27b, Tyr27d, Ser27e, Asn28, Lys30, Tyr32, and Asn34 of LCDR1; Leu50 and Asp55 of LCDR2; and Val89, Gln90, Gly91, Thr92, His93, Phe94, Pro95, and Gln96 of LCDR3 with alanine, respectively, were more than 3 times lower than that of the wild type. Therefore, these residues was determined as SDR.

Example 6: Preparation of humanized heavy chain by SDR-grafting method

A humanized heavy chain was prepared using DP7-JH4, a human heavy chain constructed by combining human immunoglobulin germline VH gene segment DP7 (Tomlinson et al., *J. Mol. Biol.*, *227*, 776-798, 1992) having an amino acid sequence similar to KR127 heavy chain variable regions and human immunoglobulin germline JH4 segment (Ravetch et al., *Cell*, *27*, 583-591 (1981)).

The Trp33 and Asn35 in HCDR1 of the KR127 were grafted into the DP7-JH4. The Met34 in HCDR1 of the KR127 is identical to that of DP7-JH4. Further, to inhibit lowering the affinity to antigen, Tyr32 in HCDR1 of the KR127 was replaced with alanine of HCDR1 of a human antibody (Gen Bank data base 75023 (SAWMN)).

The Arg50 and Tyr52 in HCDR2 of the KR127 were grafted onto the DP7-JH4. The Pro52a in HCDR2 of the KR127 is identical to that of DP7-JH4.

The Asp95, Tyr96, Arg97, Val98, and Arg102 of HCDR3 were grafted into DP7-JH4.

Further, Ala71 and Lys73 of FR 3 (framwork region 3) in the heavy chain variable region of KR127 antibody which affects the conformation of CDR loops were grafted thereto.

Then, PCR reaction was carried out using primers Ryu166 of SEQ ID NO: 23 and Hur37 of SEQ ID NO: 24 according to the method described in Example 3 to obtain a humanized heavy chain variable region gene, HuKR127VH-VII.

Ryu 166: 5'-GGA TTT GTC TGC AGT CAT TGT GGC TCT GCC CTG GAA CTT-3'
Hur 37: 5'-GAC AAA TCC ACG AGC ACA GTC TAC ATG-3'

The base sequence of the humanized heavy chain variable region gene was determined by DNA sequence analysis (Fig. 2). Then, the gene was cleaved with *EcoR*I and *Apa*I and inserted at the *EcoR*I/*Apa*I section of vector pdDdA-chKR127HC to obtain pHuKR127HC.

A humanzied antibody was prepared by combining humanized heavy chain thus obtained and the humanized antibody HZKR127I light chain described in Korean Patent No. 246128 and measured the affinity to antigen

was numbered according to the method described in Test Example 2. Humanized antibody HZKR127I was used as a control.

The affinity to antigen of the humanized antibody of about 1.5×10^{-10} M was about 50 times higher than that of HZKR127I, about 8.2×10^{-9} M.

Example 7: Preparation of humanized light chain by SDR-grafting method

A humanized light chain was prepared using DP7-JH4, a human light chian constructed by combining human immunoglobulin germline VK gene segment DPK12 (Cox et al., *Eur. J. Immunol.*, *24*, 827-836 (1994)) having an amino acid sequence similar to KR127 light chain variable regions and human immunoglobulin germline JK4 segment (Hieter et al., *J. Biol. Chem.*, *257*, 1516-1522 (1982)).

The Tyr27d, Asn28 and Asn34 in LCDR1 of KR127 were grafted into the DPK12-JK4. The amino acid residues at position 27b, 27e, 30 and 32 of DP7 is identical to those of KR127 light chain.

The Leu50 and Asp55 in LCDR2 of KR127 were grafted into the DPK12-JK4 gene.

The Val89, Gly91, Thr92, His93, Phe94, and Gln96 in LCDR3 of KR127 were grafted into the DPK12-JK4. The residues at positions 90 and 95 of DP7 is identical to those of KR127.

Further, Leu36 and Arg46 of FR 2 in the light chain variable region of KR127 antibody (which acts on interaction with heavy chain or CDR) were grafted thereto.

Then, PCR reaction was carried out using primers Ryu118 of SEQ ID NO: 25 and Ryu119 of SEQ ID NO: 26 according to the method described in Example 3 to prepare a humanized light chain variable region gene, HuKR127VH-IV.

Ryu 118: 5'-CTG TGG AGG CTG GCC TGG CTT CTG TAA TAA CCA-3'
Ryu 119: 5'-GGC CAG CCT CCA CAG CTC CTA ATC TAT CTG-3'

The base sequence of the humanized light chain variable region gene was determined by DNA sequence analysis (see HZIV of Fig. 4).. Then, the gene was cleaved with *Hind*III and *Bsi*WI and inserted at the *Hind*III/*Bsi*WI section of vector pKC-dhfr-chKR127BS to obtain pHuKR127KC.

A humanzied antibody was prepared by combining humanized light

chain thus obtained and the humanized antibody HZKR127I heavy chain described in Korean Patent No. 246128 and its affinity to antigen was measured according to the method described in Test Example 2. Humanized antibody HZKR127I was used as a control.

The affinities to antigen of the humanized antibody of about 8.4×10^{-9} M was similar to that of HZKR127I, about 8.2×10^{-9} M.

Example 8: Preparation of humanized antibody and measurement of the affinity to antigen

To prepare a plasmid containing humanized heavy chain plasmid pHuKR127HC and humanized light chain plasmid pHuKR127KC, the *Eco*RI/*Apa*I fragment containing humanized heavy chain variable region gene of pHuKR127HC and the *Hin*dIII/*Bsi*WI fragment containing humanized light chain variable region gene of pHuKR127KC were inserted at the *Eco*RI/*Apa*I and *Hin*dI/*Bsi*WI sections of vector pdCMV-dhfrC-HAV6 (KCTC 10028BP), respectively, to obtain plasmid pdCMV-dhfrC-HuKR127 (Fig. 6). *E. coli* DH5 α was transformed with the plasmid thus obtained and the transformed *E. coli* DH5α/pdCMC-dhfrC-HuKR127 was deposited on March 13, 2002 with the Korean Collection for Type Cultures(KCTC)(Address: Korea Research Institute of Bioscience and Biotechnology(KRIBB), #52, Oun-dong, Yusong-ku, Taejon, 305-333, Republic of Korea) under the accession number, KCTC 10198BP, in accordance with the terms of Budapest Treaty on the International Recognition of the Deposit of Microorganism for the Purpose of Patent Procedure.

To prepare cell line expressing the humanized antibody, dhfr-defected CHO (chinese hamster ovary) cells were transformed with plasmid pdCMV-dhfrC-HuKR127 as follows:

CHO cells (ATCC CRL 9096) were seeded to DMEM/F12 media (GIBCO) containing 10% fetal bovine serum and subcultured in an incubator at 37°C under an atmosphere of 5% CO_2. 5×10^5 cells thus obtained were seeded to the same media and incubated at 37°C overnight, followed by washing 3 times with OPTI-MEMI solution (GIBCO).

Meanwhile, 5 μg of the plasmid pdCMV-dhfrC-HuKR127 was diluted in 500 μℓ of OPTI-MEMI solution. 25 μℓ of Lipofectamine was diluted in 500 μℓ of the same solution. The resulting solutions were added

to a 15 ㎖ tube, mixed, and then, kept at room temperature for more than 15 minutes. Then, 2 ㎖ of OPTI-MEM I was added to by DNA-Lipofectamine mixture and the resulting solution was distributed evenly on the COS7 cells to be kept in a 5% CO_2 incubator at 37°C for 6 hours. Added thereto was 3 ㎖ of DMEM/F12 containing 20% fetal bovine serum and cultured for 48 hours.

Then, CHO cells were taken up with trypsin and cultured in-a-MEM media(GIBCO) of 10 % dialyzed fetal bovine serum containing G418 (GIBCO BRL, 550, ㎎/ℓ) for 2 weeks. After confirming of antibody-producing ability of the transformed clone, the clone was cultured in a-MEM media of 10 % dialyzed fetal bovine serum containing 20nM MTX to induce amplification of gene.

Cell line CHO/HuKR127 having the highest antibody-productivity was selected from the clones and deposited on March 13, 2002 with the Korean Collection for Type Cultures(KCTC) under the accession number, KCTC 10199BP, in accordance with the terms of Budapest Treaty on the International Recognition of the Deposit of Microorganism for the Purpose of Patent Procedure.

To measure the affinity to antigen of the humanized antibody HuKR127, CHO cell line thus obtained was mass cultured in a serum-absence media (CHO-SFMII, GIBCO) and subjected to protein G-shepharose 4B column (Pharmacia). Then, the antibody absorbed on the column was eluted with 0.1 M glycine solution (pH 2.7) and neutralized with 1.0 M tris solution (pH 9.0), followed by dialyzing in PBS buffer (pH 7.0). Further, the affinity to antigen of the purified antibody was determined by the competitive ELISA method described in Test Example 2 and compared with that of a control, humanized HuKR127I. The result was shown in Fig. 7.

As shown in Fig. 7, the affinity to antigen of the humanized antibody of the present invention of 1.6×10^{-10} M was about 50 times higher than 8.2×10^{-9} M3 of the control group.

Example 9: Confirmation of immune-response induction of humanized antibody

To confirm whether the humanized antibody of the present invention (HuKR127) prevents HAMA response, an analysis was conducted according to the TEPITOPE method (Sturniolo et al., *Nature Biotechnology*, *17*, 555-

561, 1999) to examine whether a peptide sequences which can bind to MHC (major histocompatibility complex) class II exists in the heavy and light chain variable regions of the humanized antibody.

Tables. 7 and 8 show the results of such analysis for MHC class II-binding peptide sequences in the heavy chain variable regions of HuKR127 and the light chain variable regions of HuKR127, respectively.

Table 7

antiboby	HzKR127I		HuKR127	
	peptide	MHC class II	peptide	MHC class II
MHC class II -binding	LVQSGAEVV	DRB1_0306 DRB1_0307 DRB1_0308 DRB1_0311 DRB1_0421 DRB1_0701 DRB1_0703	LVQSGAEVK	0
	VKPGASVKV	DRB1_0102	KKPGASVKV	0
	FSSSWMNWV	DRB1_0703	FTSAWMNWV	0
	WIGRIYPGD	DRB1_0801 DRB1_0817	WMGRIYPSG	0
	FQGKATLTA	DRB1_0401 DRB1_0402 DRB1_0405 DRB1_0408 DRB1_0421 DRB1_0426 DRB1_0801 DRB1_0802 DRB1_0804 DRB1_0806 DRB1_0813 DRB1_0817 DRB1_1101 DRB1_1102 DRB1_1104 DRB1_1106 DRB1_1114 DRB1_1120 DRB1_1121 DRB1_1128 DRB1_1302 DRB1_1305 DRB1_1307 DRB1_1311 DRB1_1321 DRB1_1322 DRB1_1323	FQGRVTMTA	DRB1_0305 DRB1_0401 DRB1_0402 DRB1_0408 DRB1_0426 DRB1_0801 DRB1_0802 DRB1_0804 DRB1_0806 DRB1_0813 DRB1_0817 DRB1_1101 DRB1_1114 DRB1_1120 DRB1_1128 DRB1_1302 DRB1_1305 DRB1_1307 DRB1_1321 DRB1_1323 DRB1_1502
	YWGQGTLVT	DRB1_0401 DRB1_0405 DRB1_0421 DRB1_0426	RWGQGTLVT	0
	IGRIYPGDG	DRB5_0101 DRB5_0105	MGRIYPSGG	DRB1_0404 DRB1_0405 DRB1_0410 DRB1_0423
	YAQKFQGKA	DRB1_0802	YAQKFQGRV	0
	VYFCAREYD	DRB1_1304	VYYCAREYR	DRB1_0301
	YWGQGTLVT	DRB1_0401 DRB1_0405 DRB1_0421 DRB1_0426	RWGQGTLVT	0
total		50		26

Table 8a

antiboby	HzkR127I		HuKR127	
	peptide	MHC class II	peptide	MHC class II
MHC class II -binding	ILMTQTPLS	DRB1_0301 DRB1_0305 DRB1_0306 DRB1_0307 DRB1_0308 DRB1_0309 DRB1_0311 DRB1_0401 DRB1_0402 DRB1_0404 DRB1_0405 DRB1_0408 DRB1_0410 DRB1_0421 DRB1_0423 DRB1_0426 DRB1_0804 DRB1_1101 DRB1_1102 DRB1_1104 DRB1_1106 DRB1_1107 DRB1_1114 DRB1_1121 DRB1_1128 DRB1_1301 DRB1_1304 DRB1_1305 DRB1_1307 DRB1_1311 DRB1_1321 DRB1_1322 DRB1_1323 DRB1_1327 DRB1_1328	IVMTQTPLS	0
	LMTQTPLSL	DRB1_0101 DRB1_0102 DRB1_1304	VMTQTPLSL	0
	WLLQKPGQS	DRB1_0101 DRB1_0305 DRB1_0309 DRB1_0401 DRB1_0408 DRB1_0421 DRB1_0426 DRB1_0802 DRB1_1101 DRB1_1107 DRB1_1114 DRB1_1120 DRB1_1128 DRB1_1302 DRB1_1305 DRB1_1307 DRB1_1321 DRB1_1323 DRB5_0101 DRB1_0105	WLLQKPGQP	0
	YYCVQGTHF	DRB1_0101 DRB1_0701 DRB1_0703 DRB5_0101 DRB5_0105	YYCVQGTHF	DRB1_0101 DRB1_0701 DRB1_0703 DRB5_0101 DRB5_0105
	YCVQGTHFP	DRB1_0401 DRB1_0421 DRB1_0426	YCVQGTHFP	DRB1_0401 DRB1_0421 DRB1_0426

Table 8b

antiboby	HzKR127I		HuKR127	
	peptide	MHC class II	peptide	MHC class II
	VGVYYCVQG	DRB1_0806	VGVYYCVQG	DRB1_0806
	IYLVSKLDS	DRB1_0301 DRB1_0305 DRB1_0306 DRB1_0307 DRB1_0308 DRB1_0309 DRB1_0311 DRB1_0405 DRB1_0410 DRB1_0801 DRB1_0802 DRB1_0804 DRB1_0806 DRB1_0813 DRB1_0817 DRB1_1101 DRB1_1102 DRB1_1104 DRB1_1106 DRB1_1107 DRB1_1114 DRB1_1120 DRB1_1121 DRB1_1128 DRB1_1301 DRB1_1302 DRB1_1304 DRB1_1305 DRB1_1307 DRB1_1311 DRB1_1321 DRB1_1322 DRB1_1323 DRB1_1327 DRB1_1328 DRB1_1501 DRB1_1506	IYLVSNRDS	DRB1_0402 DRB1_0404 DRB1_0405 DRB1_0408 DRB1_0410 DRB1_0423 DRB1_0804 DRB1_1102 DRB1_1104 DRB1_1106 DRB1_1114 DRB1_1121 DRB1_1301 DRB1_1307 DRB1_1311 DRB1_1322 DRB1_1323 DRB1_1327 DRB1_1328 DRB5_0101 DRB5_0105
	LIYLVSKLD	DRB1_0806 DRB1_1304 DRB1_1321	LIYLVSNRD	DRB1_0401 DRB1_0404 DRB1_0405 DRB1_0408 DRB1_0410 DRB1_0421 DRB1_0423 DRB1_0426 DRB1_1304
	YLVSKLDSG	0	YLVSNRDSG	DRB1_0309
total		106		40

As can be seen from Figs. 7 and 8, the number of the peptide sequence in the humanized antibody HuKR127 which binds to MHC class II was fewer than of that the HzKR127I. These results suggest that humanized antibody HuKR127 of the present invention is expected to reduce HAMA response to a greater extent than HzKR127I.

While the embodiments of the subject invention have been described and illustrated, it is obvious that various changes and modifications can be made therein without departing from the spirit of the present invention which should be limited only by the scope of the appended claims.

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSE OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT

issued pursuant to Rule 7.1

TO : HONG, Hyo Jeong
Clover Apt. 117-201, Dunsan-dong, Seo-ku, Taejon 302-772,
Republic of Korea

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: ***Escherichia coli*** **DH5@/pdCMV-dhfrC-HuKR127**	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: **KCTC 10198BP**
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: [x] a scientific description [ ] a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on **March 13 2002.**	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I above was received by this International Depositary Authority on and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: Korean Collection for Type Cultures Address: Korea Research Institute of Bioscience and Biotechnology (KRIBB) #52, Oun-dong, Yusong-ku, Taejon 305-333, Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority of authorized official(s): BAE, Kyung Sook, Director Date: **March 16 2002**

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSE OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT

issued pursuant to Rule 7.1

TO : HONG, Hyo Jeong
Clover Apt. 117-201, Dunsan-dong, Seo-ku, Taejon 302-772,
Republic of Korea

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: **CHO/HnKR127** **(CHO cell line)**	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: **KCTC 10199BP**

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:
[x] a scientific description
[ ] a proposed taxonomic designation
(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I above, which was received by it on **March 13 2002.**

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V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: Korean Collection for Type Cultures Address: Korea Research Institute of Bioscience and Biotechnology (KRIBB) #52, Oun-dong, Yusong-ku, Taejon 305-333, Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority of authorized official(s): Bae K.S. BAE, Kyung Sook, Director Date: **March 16 2002**

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Form BP/4 (KCTC Form 17)

What is claimed is :

1. A process for preparing a humanized antibody comprising the steps of:

(a) selecting a specificity determining residue (SDR) of the complementarity determining region (CDR) of murine monoclonal antibody heavy chain and light chain variable regions; and

(b) grafting said SDR to at least one of the corresponding amino acid sequences into human antibody variable regions.

2. The process of claim 1, wherein step (a) is conducted by replacing each the amino acid residues of CDR with alanine to produce transformants, selecting a transformant that has lower affinity to the human antigen (K_D) than the original murine antibody and determining the replaced amino acid residue of said transformant as an SDR.

3. The process of claim 2, wherein the CDR is selected from the group consisting of HCDR1(aa 31-35), HCDR2(aa 50-65) and HCDR3(aa 95-102) of the heavy chain (SEQ ID NO: 2); and LCDR1(aa 24-34), LCDR2(aa 50-56) and LCDR3(aa 89-97) of the light chain (SEQ ID NO: 4) of the murine monoclonal antibody variable regions of hepatitis B virus pre-S1 antigen, selecting a transformant that has an affininty to antigen which is more than 3 times lower than the original murine antibody when replaced with alanine, determining the replaced amino acid residue of said transformant as an SDR, and grafting said SDR to the corresponding amino acid sequence in human antibody heavy chain and light chain

4. The process of claim 3, which is characterized in that the at least one of Trp33, Met34, and Asn35 of HCDR1; Arg50, Tyr52, and Pro52a of HCDR2; and Glu95, Tyr96, and Glu98 of HCDR3 of the murine monoclonal antibody KR127 heavy chain, is grafted to the corresponding amino acid sequences in human antibody heavy chain.

5. The process of claim 4, which is characterized in that the at least one of the following grafting steps is carried out:

(a) the amino acid residue at position 32 in HCDR1 of human

antibody with alanine;

(b) the amino acid residue at position 97 in HCDR3 of human antibody with arginine or alanine;

(c) the amino acid residue at position 98 in HCDR3 of human antibody with valine; and

(d) the amino acid residue at position 102 in HCDR3 of human antibody with arginine or alanine.

6. The process of claim 5, which is characterized in that the at least one of Trp33 and Asn35 of HCDR1; Arg50 and Tyr52 of HCDR2; and Arg95 and Tyr96 of HCDR3 of the murine monoclonal antibody KR127 heavy chain, is grafted into the human antibody heavy chain DP7-JH4.

7. The process of claim 6, which is characterized in that the amino acid residues of the Ala71 and Lys73 in Framework region 3 of the murine monoclonal antibody KR127 heavy chain variable region, of further grafted into the human antibody heavy chain DP7-JH4.

8. The process of claim 3, which is characterized in that the at least one of the Leu27b, Tyr27d, Ser27e, Asn28, Lys30, Tyr32 and Asn34 of LCDR1; Leu50 and Asp55 of LCDR2; and Val89, Gln90, Gly91, Thr92, His93, Phe94, Pro95, and Gln96 of LCDR3 of the murine monoclonal antibody KR127 light chain, is grafted into the human antibody light chain.

9. The process of claim 8, which is characterized in that the Tyr27d, Asn28, Asn34 of LCDR1; Leu50 and Asp55 of LCDR2; and Val89, Gly91, Thr92, His93, Phe94, Pro95, and Gln96 of LCDR3 of the murine monoclonal antibody KR127 light chain, is grafted into the human antibody light chain DPH12-JK4.

10. The process of claim 8, which is characterized in that the Leu36 and Arg46 in Framework region 2 of the murine monoclonal antibody KR127 light chain variable region, are further grafted into the human antibody light chain DPH12-JK4.

11. A humanized antibody prepared by the process of any one of

claims 1 to 10, which has an affinity to antigen of higher than 8.2×10^{-9} M and suppresses HAMA (human anti-mouse antibody) response to a greater extent than an antibody prepared according to CDR-grafting method.

12. The humanized antibody of claim 11, which has the amino acid sequence of SEQ ID NO: 2 for the heavy chain variable region of HBV pre-S1 antigen.

13. The humanized antibody of claim 11, which has the amino acid sequence of SEQ ID NO: 4 for the light chain variable region of HBV pre-S1 antigen.

14. The humanized antibody of any one of claims 11 to 13, which is produced by CHO/HuKR127 (Accession No.: KCTC 10199BP).

15. A DNA encoding the humanized antibody heavy chain containing the amino acid sequence of SEQ ID NO: 2 for the heavy chain variable region of HBV pre-S1 antigen.

16. The DNA of claim 15, wherein the variable region has the nucleotide sequence of SEQ ID NO: 1.

17. A DNA encoding the humanized antibody light chain containing the amino acid sequence of SEQ ID NO: 4 for the light chain variable region of HBV pre-S1 antigen.

18. The DNA of claim 17, wherein the variable region has the nucleotide sequence of SEQ ID NO: 3.

19. An expression vector pHuKR127HC comprising the DNA of claim 16 for expressing the humanized antibody heavy chain for HBV pre-S1 antigen.

20. An expression vector pHuKR127KC comprising the DNA of claim 18 for expressing the humanized antibody light chain for HBV pre-S1 antigen.

21. An expression vector pdCMV-dhfrC-HuKR127 comprising both the DNAs of claim 16 and 18 for expressing the humanized antibody light and heavy chains for HBV pre-S1 antigen.

22. An *E. coli* DH5α/pdCMV-dhfrC-HuKR127 (Accession No.: KCTC 10198BP) transformed with the expression vector of claim 21.

23. CHO cell line CHO/HuKR127 (Accession No.: KCTC 10199BP) producing the humanized antibody of claim 11.

24. A composition for preventing or treating HBV infection comprising the humanized antibody of any one of claims 11 to 13.

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FIG. 1

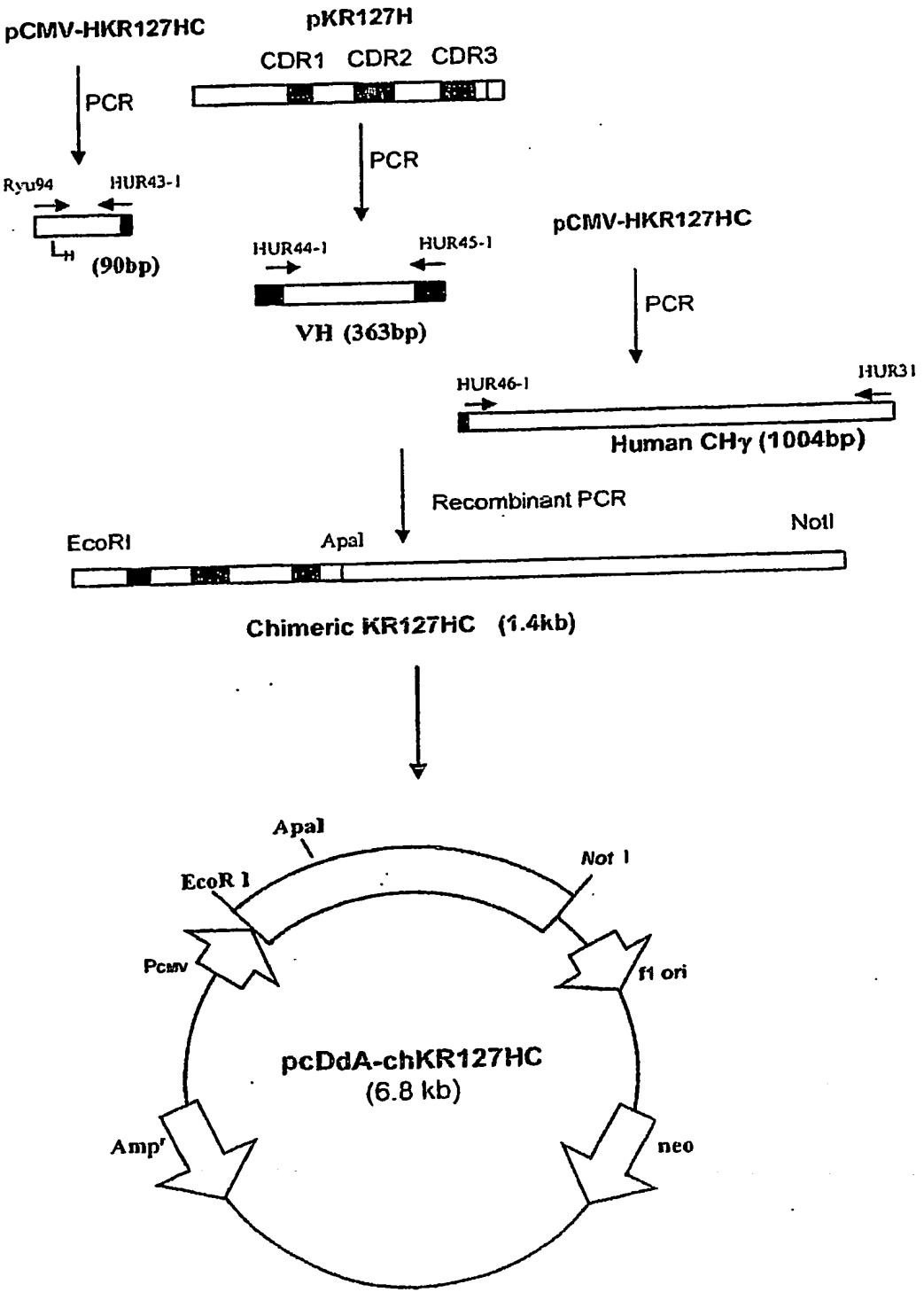
pCMV-HKR127HC
pKR127H
CDR1 CDR2 CDR3
PCR
PCR
Ryu94
HUR43-1
L_H
(90bp)
HUR44-1
HUR45-1
VH (363bp)
pCMV-HKR127HC
PCR
HUR46-1
HUR31
Human CHγ (1004bp)
Recombinant PCR
EcoRI
ApaI
NotI
Chimeric KR127HC (1.4kb)
ApaI
EcoR I
Not I
Pcmv
f1 ori
pcDdA-chKR127HC
(6.8 kb)
Amp^r
neo

FIG. 2A

```
                 Q  V  Q  L  Q  Q  S  G  P  E  L  V  K  P
KR127VH    CAG GTC CAG CTG CAG CAG TCT GGA CCT GAA CTG GTG AAG CCT 42
                 Q  V  Q  L  V  Q  S  G  A  E  V  K  K  P
DP7        CAG GTG CAG CTG GTG CAG TCT GGG GCT GAG GTG AAG AAG CCT
HZ I       CAG GTC CAG CTG GTG CAG TCT GGA GCT GAA GTG GTG AAG CCT
HZ VII     CAG GTC CAG CTG GTG CAG TCT GGA GCT GAA GTG AAG AAG CCT
HZ I        -  -  -  -  V  -  -  -  A  -  V  -  -  -
HZ VII      -  -  -  -  V  -  -  -  A  -  V  K  -  -

                 G  A  S  V  K  I  S  C  K  A  S  G  Y  A
KR127VH    GGG GCC TCA GTG AAG ATT TCC TGC AAA GCT TCT GGC TAC GCA 84
                 G  A  S  V  K  V  S  C  K  A  S  G  Y  T
DP7        GGG GCC TCA GTG AAG GTT TCC TGC AAG GCA TCT GGA TAC ACC
HZI        GGG GCC TCA GTG AAG GTT TCC TGC AAA GCT TCT GGC TAC GCA
HZ VII     GGG GCC TCA GTG AAG GTT TCC TGC AAA GCT TCT GGC TAC ACC
HZI         -  -  -  -  -  V  -  -  -  -  -  -  -  T
HZVII       -  -  -  -  -  V  -  -  -  -  -  -  -  -

                                CDR1
                 F  S  S  S  W  M  N  W  V  K  Q  R  P  G
KR127VH    TTC AGT AGT TCT TGG ATG AAC TGG GTG AAG CAG AGG CCT GGA 126
                 F  T  S  Y  Y  M  H  W  V  R  Q  A  P  G
DP7        TTC ACC AGC TAC TAT ATG CAC TGG GTG CGA CAG GCC CCT GGA
HZI        TTC AGT AGT TCT TGG ATG AAC TGG GTG CGA CAG GCC CCT GGA
HZ VII     TTC ACC AGT GCT TGG ATG AAC TGG GTG CGA CAG GCC CCT GGA
HZI         -  -  -  -  -  -  -  -  -  R  -  A  -  -
HZVII       -  T  -  A  -  -  -  -  -  R  -  A  -  -

                                                CDR2
                 Q  G  L  E  W  I  G  R  I  Y  P  G  D  G
KR127VH    CAG GGT CTT GAG TGG ATT GGA CGG ATT TAT CCT GGA GAT GGA 168
                 Q  G  L  E  W  M  G  I  I  N  P  S  G  G
DP7        CAA GGG CTT GAG TGG ATG GGA ATA ATC AAC CCT AGT GGT GGT
HZI        CAG GGT CTT GAG TGG ATT GGA CGG ATT TAT CCT GGA GAT GGA
HZVII      CAG GGT CTT GAG TGG ATG GGA CGG ATT TAT CCT AGT GGT GGA
HZI         -  -  -  -  -  -  -  -  -  -  -  -  -  -
HZVII       -  -  -  -  -  M  -  -  -  -  -  S  G  -
```

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FIG. 2B

CDR2

	D	T	N	Y	N	G	K	F	K	G	K	A	T	L	
KR127VH	GAT	ACT	AAC	TAC	AAT	GGG	AAG	TTC	AAG	GGC	AAG	GCC	ACA	CTG	210
	S	T	S	Y	A	Q	K	F	Q	G	R	V	T	M	
DP7	AGC	ACA	AGC	TAC	GCA	CAG	AAG	TTC	CAG	GGC	AGA	GTC	ACC	ATG	
HZ1	GAT	ACT	AAC	TAC	GCA	CAG	AAG	TTC	CAG	GGC	AAG	GCC	ACA	CTG	
HZV11	AGC	ACT	AGC	TAC	GCA	CAG	AAG	TTC	CAG	GGC	AGA	GTC	ACA	ATG	
HZ1	-	-	-	-	A	Q	-	-	Q	-	-	-	-	-	
HZV11	S	-	S	-	A	Q	-	-	Q	-	R	V	-	M	

	T	A	D	K	S	S	S	T	A	Y	M	Q	L	S	
KR127VH	ACT	GCA	GAC	AAA	TCC	TCC	AGC	ACA	GCC	TAC	ATG	CAG	CTC	AGC	252
	T	R	D	T	S	T	S	T	V	Y	M	E	L	S	
DP7	ACC	AGG	GAC	ACG	TCC	ACG	AGC	ACA	GTC	TAC	ATG	GAG	CTG	AGC	
HZ1	ACT	GCA	GAC	AAA	TCC	ACG	AGC	ACA	GCC	TAC	ATG	GAG	CTC	AGC	
HZV11	ACT	GCA	GAC	AAA	TCC	ACG	AGC	ACA	GTC	TAC	ATG	GAG	CTC	AGC	
HZ1	-	-	-	-	-	T	-	-	-	-	-	E	-	-	
HZV11	-	-	-	-	-	T	-	-	V	-	-	E	-	-	

	S	L	T	S	V	D	S	A	V	Y	F	C	A	R	
KR127VH	AGC	CTG	ACC	TCT	GTG	GAC	TCT	GCG	GTC	TAT	TTC	TGT	GCA	AGA	294
	S	L	R	S	E	D	T	A	V	Y	Y	C	A	R	
DP7	AGC	CTG	AGA	TCT	GAG	GAC	ACG	GCC	GTG	TAT	TAC	TGT	GCG	AGA	
HZ1	AGC	CTG	AGA	TCT	GAG	GAC	ACG	GCG	GTC	TAT	TTC	TGT	GCA	AGA	
HZV11	AGC	CTG	AGA	TCT	GAG	GAC	ACG	GCG	GTG	TAT	TAC	TGT	GCA	AGA	
HZ1	-	-	R	-	E	-	T	-	-	-	-	-	-	-	
HZV11	-	-	R	-	E	-	T	-	-	-	Y	-	-	-	

CDR3

	E	Y	D	E	A	Y	W	G	Q	G	T	L	V	T	
KR127VH	GAG	TAC	GAC	GAG	GCT	TAC	TGG	GGC	CAA	GGG	ACT	CTG	GTC	ACT	336
							W	G	Q	G	T	L	V	T	
HZ1	GAG	TAC	GAC	GAG	GCT	TAC	TGG	GGC	CAA	GGA	ACT	CTG	GTC	ACT	
HZV11	GAG	TAC	CGG	GTT	GCC	CGT	TGG	GGC	CAA	GGA	ACT	CTG	GTC	ACT	
HZ1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
HZV11	-	-	R	V	-	R	-	-	-	-	-	-	-	-	

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FIG. 2C

	V	S	A	
KR127VH	GTC	TCT	GCA	345
	V	S	S	
HZI	GTC	TCT	TCA	
HZVII	GTC	TCT	TCA	
HZI	-	-	S	
HZVII	-	-	S	

FIG. 3

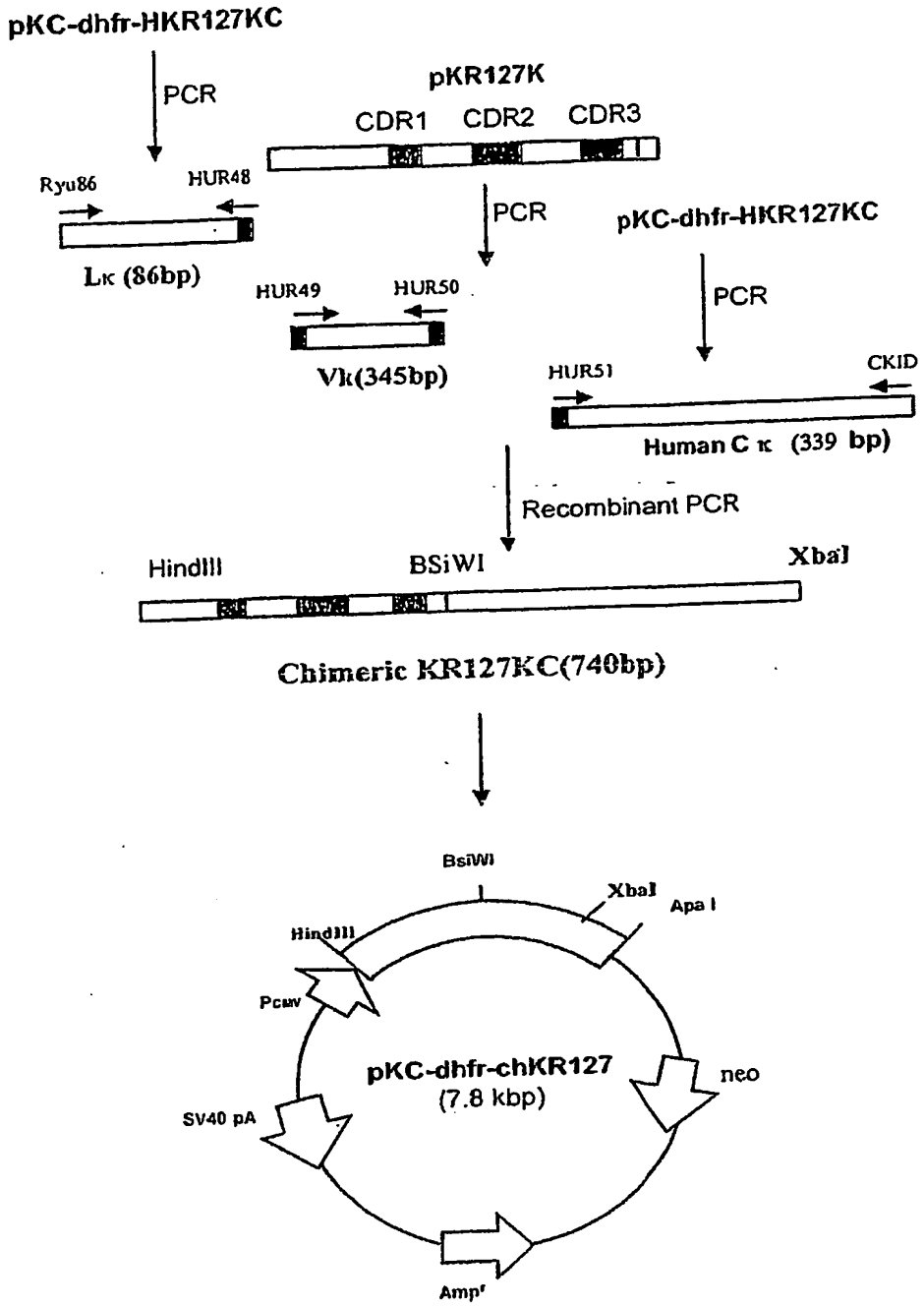
pKC-dhfr-HKR127KC
PCR
pKR127K
CDR1 CDR2 CDR3
Ryu86
HUR48
Lκ (86bp)
PCR
pKC-dhfr-HKR127KC
HUR49
HUR50
Vk(345bp)
PCR
HUR51
CKID
Human C κ (339 bp)
Recombinant PCR
HindIII
BSiWI
XbaI
Chimeric KR127KC(740bp)
BsiWI
XbaI
Apa I
HindIII
Pcmv
pKC-dhfr-chKR127
(7.8 kbp)
neo
SV40 pA
Ampr

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FIG. 4A

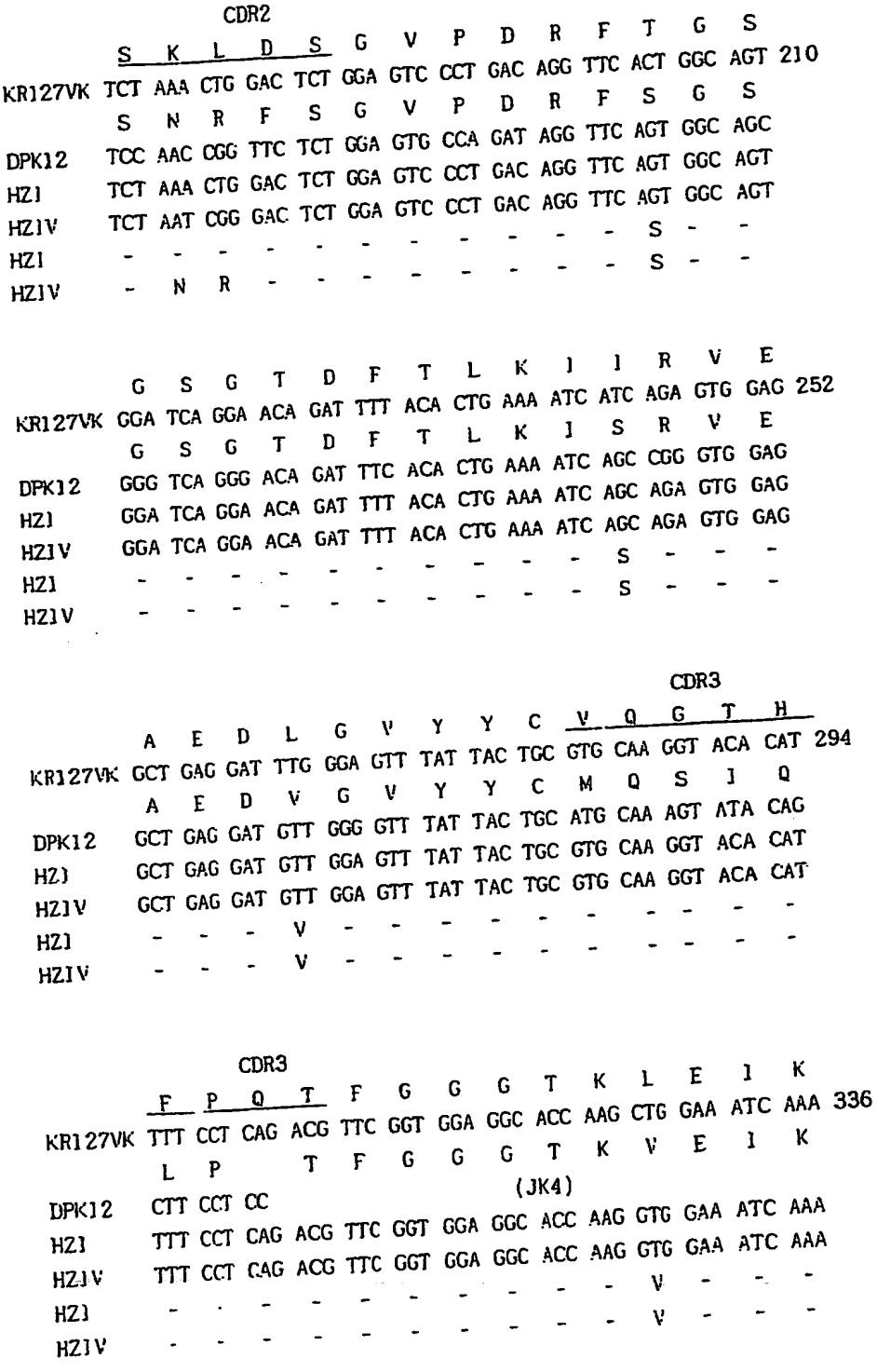
```
                D  I  L  M  T  Q  T  P  L  I  L  S  V  T
KR127VK  GAT ATC TTG ATG ACC CAA ACT CCA CTT ATT TTG TCG GTT ACC 42
                D  I  V  M  T  Q  T  P  L  S  L  S  V  T
DPK12    GAT ATT GTG ATG ACC CAG ACT CCA CTC TCT CTG TCC GTC ACC
HZ1      GAT ATC TTG ATG ACC CAA ACT CCA CTT TCT TTG TCG GTT ACC
HZ1V     GAT ATC GTG ATG ACC CAA ACT CCA CTT TCT TTG TCG GTT ACC
HZ1      -  -  -  -  -  -  -  -  -  S  -  -  -  -
HZ1V     -  -  V  -  -  -  -  -  -  S  -  -  -  -

                                          CDR1
                I  G  Q  P  A  S  I  S  C  K  S  S  Q  S
KR127VK  ATT GGA CAA CCA GCC TCT ATC TCT TGC AAG TCA AGT CAG AGC 84
                P  G  Q  P  A  S  I  S  C  K  S  S  Q  S
DPK12    CCT GGA CAG CCG GCC TCC ATC TCC TGC AAG TCT AGT CAG AGC
HZ1      CCT GGA CAA CCA GCC TCT ATC TCT TGC AAG TCA AGT CAG AGC
HZ1V     CCT GGA CAA CCA GCC TCT ATC TCT TGC AAG TCA AGT CAG AGC
HZ1      P  -  -  -  -  -  -  -  -  -  -  -  -  -
HZ1V     P  -  -  -  -  -  -  -  -  -  -  -  -  -

                                CDR1
                L  L  Y  S  N  G  K  T  Y  L  N  W  L  L
KR127VK  CTC TTA TAT AGT AAT GGA AAA ACC TAT TTG AAT TGG TTA TTA 126
                L  L  H  S  D  G  K  T  Y  L  Y  W  Y  L
DPK12    CTC CTG CAT AGT GAT GGA AAG ACC TAT TTG TAT TGG TAC CTG
HZ1      CTC TTA TAT AGT AAT GGA AAA ACC TAT TTG AAT TGG TTA TTA
HZ1V     CTC TTA TAT AGT AAT GGA AAA ACC TAT TTG AAT TGG TTA TTA
HZ1      -  -  -  -  -  -  -  -  -  -  -  -  -  -
HZ1V     -  -  -  -  -  -  -  -  -  -  -  -  -  -

                                                CDR2
                Q  R  P  G  Q  S  P  K  R  L  I  Y  L  V
KR127VK  CAG AGG CCA GGC CAG TCT CCA AAG CGC CTA ATC TAT CTG GTG 168
                Q  K  P  G  Q  P  P  Q  L  L  I  Y  E  V
DPK12    CAG AAG CCA GGC CAG CCT CCA CAG CTC CTG ATC TAT GAA GTT
HZ1      CAG AAG CCA GGC CAG TCT CCA AAG CGC CTA ATC TAT CTG GTG
HZ1V     CAG AAG CCA GGC CAG CCT CCA CAG CGC CTA ATC TAT CTG GTG
HZ1      -  K  -  -  -  -  -  -  -  -  -  -  -  -
HZ1V     -  K  -  -  -  P  -  Q  -  -  -  -  -  -
```

FIG. 4B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KR127VK TCT AAA CTG GAC TCT GGA GTC CCT GAC AGG TTC ACT GGC AGT 210
S N R F S G V P D R F S G S
DPK12 TCC AAC CGG TTC TCT GGA GTG CCA GAT AGG TTC AGT GGC AGC
HZ1 TCT AAA CTG GAC TCT GGA GTC CCT GAC AGG TTC AGT GGC AGT
HZ1V TCT AAT CGG GAC TCT GGA GTC CCT GAC AGG TTC AGT GGC AGT
HZ1 - - - - - - - - - - S - -
HZ1V - N R - - - - - - - S - -
G S G T D F T L K I I R V E
KR127VK GGA TCA GGA ACA GAT TTT ACA CTG AAA ATC ATC AGA GTG GAG 252
G S G T D F T L K I S R V E
DPK12 GGG TCA GGG ACA GAT TTC ACA CTG AAA ATC AGC CGG GTG GAG
HZ1 GGA TCA GGA ACA GAT TTT ACA CTG AAA ATC AGC AGA GTG GAG
HZ1V GGA TCA GGA ACA GAT TTT ACA CTG AAA ATC AGC AGA GTG GAG
HZ1 - - - - - - - - - - S - - -
HZ1V - - - - - - - - - - S - - -
CDR3
A E D L G V Y Y C V Q G T H
KR127VK GCT GAG GAT TTG GGA GTT TAT TAC TGC GTG CAA GGT ACA CAT 294
A E D V G V Y Y C M Q S I Q
DPK12 GCT GAG GAT GTT GGG GTT TAT TAC TGC ATG CAA AGT ATA CAG
HZ1 GCT GAG GAT GTT GGA GTT TAT TAC TGC GTG CAA GGT ACA CAT
HZ1V GCT GAG GAT GTT GGA GTT TAT TAC TGC GTG CAA GGT ACA CAT
HZ1 - - - V - - - - - - - - - -
HZ1V - - - V - - - - - - - - - -
CDR3
F P Q T F G G G T K L E I K
KR127VK TTT CCT CAG ACG TTC GGT GGA GGC ACC AAG CTG GAA ATC AAA 336
L P T F G G G T K V E I K
DPK12 CTT CCT CC (JK4)
HZ1 TTT CCT CAG ACG TTC GGT GGA GGC ACC AAG GTG GAA ATC AAA
HZ1V TTT CCT CAG ACG TTC GGT GGA GGC ACC AAG GTG GAA ATC AAA
HZ1 - - - - - - - - - - V - - -
HZ1V - - - - - - - - - - V - - -

FIG. 5

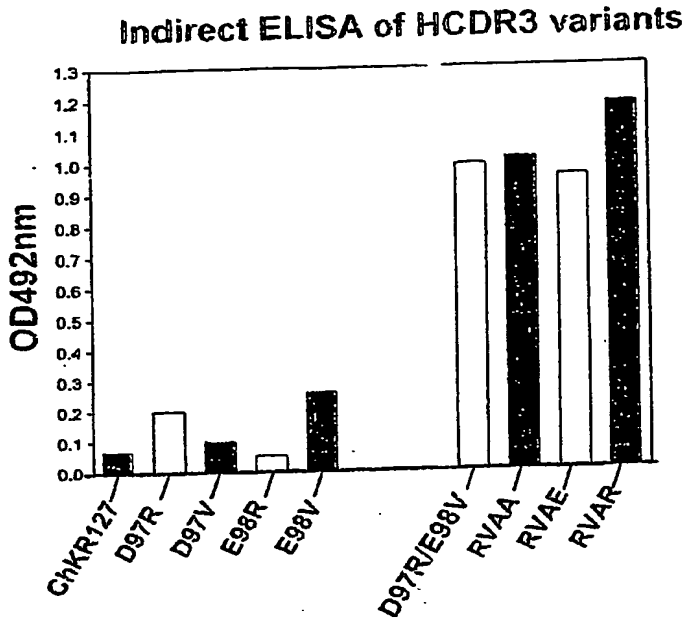
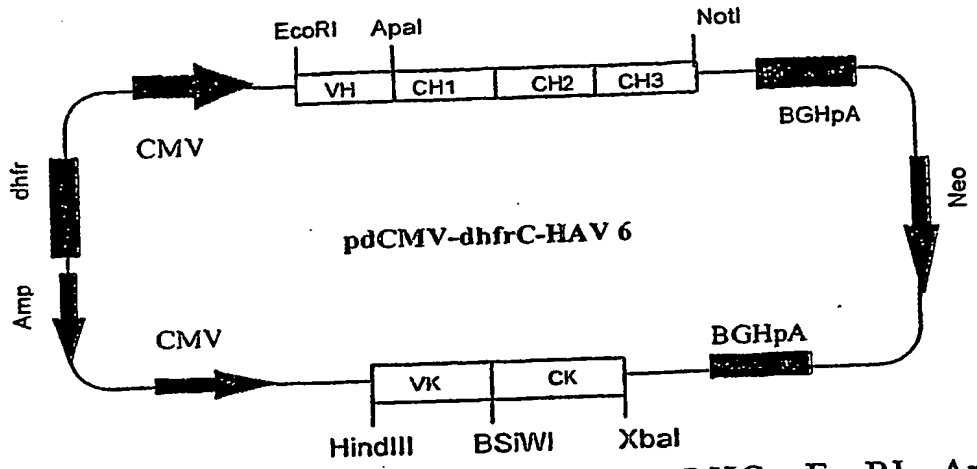


FIG. 6



pHu KR127 HC - EcoRI - ApaI fragment
subcloning
pHuKR127KC - HindIII - BSiWI fragment
subcloning

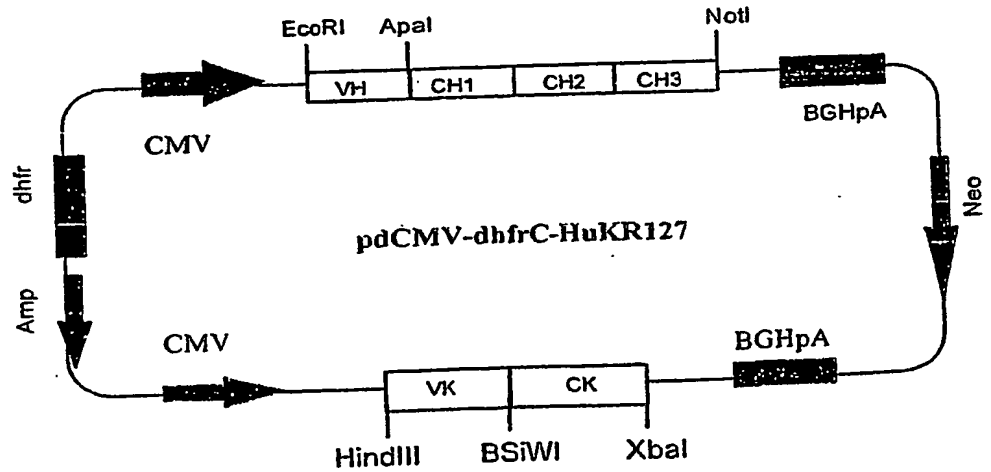
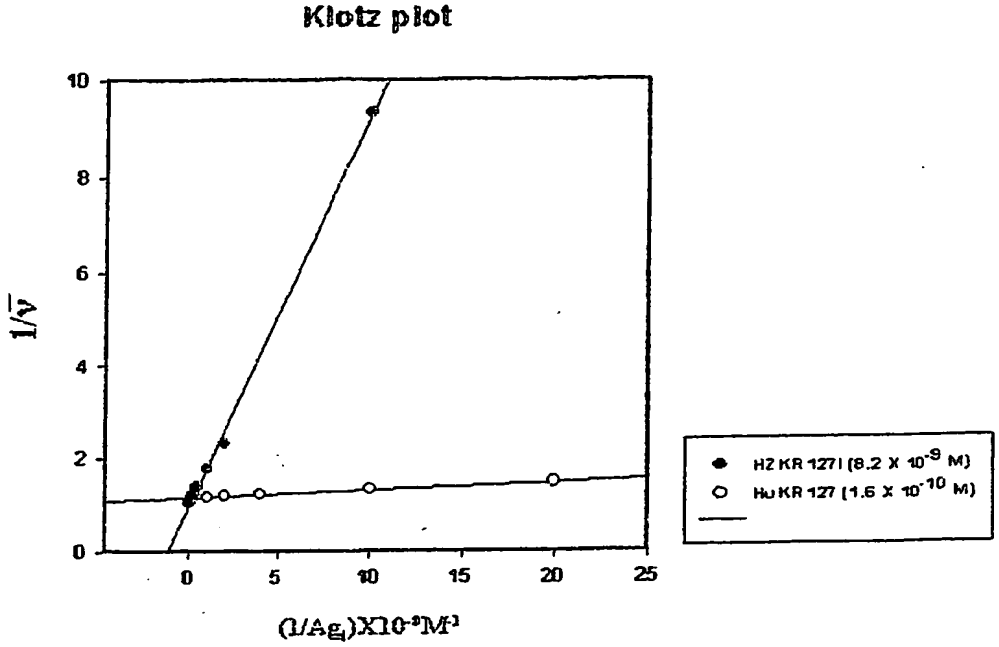


FIG. 7


Klotz plot
10
8
6
4
2
0
1/v̄
0
5
10
15
20
25
(1/Ag)X10⁻⁹M⁻¹
Hz KR 1271 (8.2 X 10⁻⁹ M)
Hu KR 127 (1.6 X 10⁻¹⁰ M)

Sequence Listing

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cctggacagg gtcttgagtg gatgggacgg atttatccta gtggtggaag cactagctac 180

gcacagaagt tccagggcag agtcacaatg actgcagaca aatccacgag cacagtctac 240

atggagctca gcagcctgag atctgaggac acggccgtgt attactgtgc aagagagtac 300

cgggttgccc gttggggcca aggaactctg gtcactgtct cttca 345

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1 5 10 15

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20 25 30

Trp Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Tyr Pro Ser Gly Gly Ser Thr Ser Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Met Thr Ala Asp Lys Ser Thr Ser Thr Val Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Tyr Arg Val Ala Arg Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ala
115

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atctcttgca agtcaagtca gagcctctta tatagtaatg gaaaaaccta tttgaattgg 120

ttattacaga agccaggcca gcctccacag cgcctaatct atctggtgtc taatcgggac 180

tctggagtcc ctgacaggtt cagtggcagt ggatcaggaa cagattttac actgaaaatc 240

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20 25 30

Asn Gly Lys Thr Tyr Leu Asn Trp Leu Leu Gln Lys Pro Gly Gln Pro
35 40 45

Pro Gln Arg Leu Ile Tyr Leu Val Ser Asn Arg Asp Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
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Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Val Gln Gly
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Thr His Phe Pro Gln Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
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<213> Artificial Sequence

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<212> DNA
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<223> HUR31

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<213> Artificial Sequence

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<223> Ryu 119

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<213> Artificial Sequence

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cctggacagg gtcttgagtg gattggacgg atttatcctg gagatggaga tactaactac 180

aatgggaagt tcaagggcaa ggccacactg actgcagaca aatcctccag cacagcctac 240

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1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Ser
20 25 30

Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Val Asp Ser Ala Val Tyr Phe Cys
85 90 95

Ala Arg Glu Tyr Asp Glu Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ala
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tctggagtcc ctgacaggtt cactggcagt ggatcaggaa cagattttac actgaaaatc 240

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1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
20 25 30

Asn Gly Lys Thr Tyr Leu Asn Trp Leu Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
50 55 60

Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
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Ile Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Val Gln Gly
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cctggacaag ggcttgagtg gatgggaata atcaacccta gtggtggtag cacaagctac 180

gcacagaagt tccagggcag agtcaccatg accagggaca cgtccacgag cacagtctac 240

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Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Ile Ile Asn Pro Ser Gly Gly Ser Thr Ser Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
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85 90 95

Ala Arg

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tctggagtgc cagataggtt cagtggcagc gggtcaggga cagatttcac actgaaaatc 240

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cc 302

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1 5 10 15

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20 25 30

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35 40 45

Pro Gln Leu Leu Ile Tyr Glu Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ser
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Ile Gln Leu Pro
100

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gcacagaagt tccagggcaa ggccacactg actgcagaca aatccacgag cacagcctac 240

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gacgaggctt actggggcca aggaactctg gtcactgtct cttca 345

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Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Val Lys Pro Gly Ala

1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Ser
20 25 30

Trp Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile Tyr Pro Gly Asp Gly Ser Thr Ser Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe Cys
85 90 95

Ala Arg Glu Tyr Asp Glu Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 37
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<213> Artificial Sequence

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<223> LIGHT CHAIN of HZI

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ttattacaga agccaggcca gtctccaaag cgcctaatct atctggtgtc taaactggac 180

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<400> 38

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Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
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Asn Gly Lys Thr Tyr Leu Tyr Trp Leu Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Val Gln Gly
85 90 95

Thr His Phe Pro Gln Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR03/00564

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C07K 16/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7 C07K 16/18, C07K 16/08, C12N 15/13, C12N 15/51, C12N 15/62

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean Patents and applications for inventions since 1975

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

NCBI PubMed database, Esp@cenet database "specificity-determining residues or humanized monoclonal antibody"

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y A	TAMURA M. et al. 'Structural correlates of an anticarcinoma antibody: Identification of specificity-determining residues (SDRs) and development of a minimally immunogenic antibody variant by retention of SDRs only' In: J Immunol, 2000, Vol.164, pp1432-1441 See the whole document	1, 2, 11 3, 24 4-10, 12-23
Y A	KR 2000-0033008 A (KOREA GREEN CROSS CORP. & KIST) 15 June 2000 (15.06.2000) See the whole document	1-3, 24 4-23
A	KR 1999-008649 A (KIST) 5 February 1999 (05.02.1999) See the claims	1-24
A	KR 1999-008650 A (KIST) 5 February 1999 (05.02.1999) See the claims	1-24
A	IWAHASHI M. et al. 'CDR substitutions of a humanized monoclonal antibody (CC49): contributions of individual CDRs to antigen binding and immunogenicity' In: Mol Immunol, 1999, Vol.36, pp1079-1091 See the whole document	1-24

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 JUNE 2003 (28.06.2003)	30 JUNE 2003 (30.06.2003)

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR03/00564

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
KR 2000-0033008 A	15.06.2000	None	
KR 1999-008649 A	05.02.1999	None	
KR 1999-008650 A	05.02.1999	None	